BONE MARROW BIOPSY

Hæmatology in the Light of
Sternal Puncture

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Hæmatology in the Light of Sterna Puncture

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WITH 7 PLATES (6 IN COLOUR) AND 191 TEXT-FIGURES



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TRANSLATORS' PREFACE

MANY papers and reviews on sternal marrow biopsy findings have appeared in medical journals in many languages, yet until Leitner's monograph was published no comprehensive work on this subject existed. The need for a reference work of this kind has been felt by all hæmatologists, clinicians and pathologists, and the senior translator was, in fact, working on such a volume when he was asked to review Leitner's book. This book covered the ground very well indeed and particularly with regard to the Continental literature, so it was considered that a translation, with the addition of later references and the inclusion of those English and American papers which had not been included owing to war difficulties, would be the most satisfactory course to follow.

Leitner has given the translators wide discretion in the incorporation of new material, and has made available his additions and alterations for the second Swiss edition, so that the book can be considered up to date at the time of going to press. Leitner's discussion on cell morphology, on the interpretation of sternal marrow findings and on the ætiology, diagnosis, prognosis and treatment of disorders affecting the hæmopoietic system has been left intact. Though we agree with Leitner on most cardinal points, there are necessarily points of difference between the Swiss and the Anglo-American schools of medicine. Continental hæmatology is more exclusively morphological and lays great emphasis on the study of the appearance of red cells relying largely on Price-Jones curves and the colour index. British and American hæmatologists utilize volumetric methods more commonly, and stress the value of the hæmatocrit, developed chiefly by Wintrobe, in the investigation of the anæmias.

In this country, the nucleated cells, both red and white, in the myelogram are recorded in percentages, and most workers follow Turnbull's nomenclature for the red series, but Leitner expresses the nucleated red cells per 100 white cells, and in his classification uses a modified Naegeli's terminology. For the convenience of readers the temperature readings have been converted from centigrade to Fahrenheit, and the hæmoglobin recorded in percentages and grams per 100 ml. of blood.

The section on acute erythræmic myelosis has been largely rewritten and a new case history has been included, while most parts of the book have had the results of more recent work added to them

We believe that this book will provide English-speaking colleagues interested in hæmatology, with an adequate and reliable reference book on bone marrow biopsy.

We would like to express our thanks to Mrs. Neumark for assistance with the bibliography, to Professor W. D. Newcomb and Dr. G. K. C. Rettie for advice and criticism, and to Miss B. Camkin, Miss H. Hamilton and Mrs. M. Reynolds for secretarial help

C. J. C. B
E N.

AUTHOR'S PREFACE

This monograph is based on investigations carried out at the medical clinic of the University of Berne (Director: Professor W. Frey), and first published in the Folia Hæmatologica (Lpz.) in 1941. The requests of many colleagues have suggested the need for a new edition and each chapter has been thoroughly revised while preserving the clinical outlook on marrow biopsy. The amount of material investigated has increased considerably since the previous publication and the wider use of sternal puncture in clinical diagnosis has produced many important papers, revealing many new facts. Every effort has been made to review the literature of the subject critically and fully, and to compare it with my own findings in the different diseases.

This book is mainly intended for practical clinical use. It is, however, necessary to discuss many important hæmatological problems as sternal marrow biopsy cannot be divorced from other investigations and from clinical findings, if it is to be fruitful for the elucidation of individual cases. Illustrative cases are quoted with short histories and a summary of the most important points is appended to each section.

The publication of this monograph has been facilitated by the courtesy of the Akademische Verlagsgesellschaft *Leipzig*, who have allowed me to use the blocks from my previous paper, but the number of photo-micrographs has been considerably increased. In order to make the book useful for both clinic and laboratory, the table of the origin of the various blood cells and the marrow pictures of the most important diseases have been reproduced as plates, mostly in colour. The coloured plates have been prepared with great care and skill by Miss Helene Gautschi from original preparations, and I should like to express my thanks to her. I owe special thanks to the publishers, Messrs. Benno Schwabe and Co. Basle, for their consideration and their skill in producing such a fine volume.

S. J. LEITNER.

HEILIGENSCHWENDI, BERNE.

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PLATES

CHAPTER I

INTRODUCTORY AND HISTORICAL

NUCLEATED red blood corpuscles were first observed in the bone marrow by Neumann in 1868. He also discovered the transformation of fatty marrow into red hæmopoietic marrow in anæmia Naegeli pointed out the part played by the marrow in leucopoiesis and leucocytosis while Schilling, by his comprehensive studies, showed the importance of the clinical examination of the blood in physiology and pathology. The value of morphological examinations of peripheral blood has been universally acknowledged since the work of Ehrlich, Naegeli, Schilling and Arneth Naegeli and Schilling soon began to link up the reactions of the peripheral blood with changes at the site of origin of the blood cells, viz., the bone marrow. It may be mentioned incidentally that during the last few years the origin of the lymphocytes also has been studied by the examination of material obtained by puncture of the lymph glands and by biopsy (Forkner, 1927 ; Pavlowsky, 1934 ; Introzzi, 1935 ; Weil *et al* , 1938 ; Stahel, 1939 ; Leitner, 1940 ; Tischendorf, 1942 ; and others)

Studies of bone marrow have not only produced new facts for clinical work, especially in the field of hæmatology, but have also made exact diagnosis possible in many cases. Investigations at first depended on post-mortem material, but this was found to be of limited value only, owing to the rapidity of the degenerative changes in the marrow cells which take place after death, first described by Schilling and his collaborators (Bantz, 1922 ; Yamamoto, 1925) First attempts at marrow biopsy by trephining the epiphysis of the femur (Pianese, 1905) or the tibia (Ghedini, 1910) were too much in the nature of major surgical procedures, and therefore could not become popular as aids to clinical diagnosis. Nevertheless, several studies were made by trephining the tibia, notably by Spuler and Schittenhelm (1913), Zadek (1921), Morris and Falconer (1922), and Peabody (1927) Seyfarth in 1923 first suggested trephining the sternum This much easier procedure found wider application and was used successfully by Schilling (1925) and afterwards by Weiner and Kaznelson (1926), Barta (1931), Escudero and Varela (1932), Custer (1933) and Dameshek (1935)

The use of marrow biopsy in clinical work was, however, made possible only after the introduction of sternal puncture by Arinkin in 1929 This was rapidly followed by intensive investigations of the morphology and function of the marrow, and eventually established marrow biopsy as a useful diagnostic

method in clinical practice. (Schilling, 1928; Nordenson, 1935; Segerdahl, 1935; Introzzi, 1935; Schulten, 1936; Mallarmé, 1936; Rohr, 1937–40; Picena, 1937; Klima, 1938; Weil and Perlès, 1938, Alder, 1939; Henning and Keilhack, 1939; de Weerdt, 1939; Fieschi, 1940; Leitner, 1940–41; Markoff, 1936, 1939; Thaddea, 1943; Kienle, 1943). The diagnostic possibilities of sternal puncture in many common and rare blood disorders and in internal diseases, such as tumours and infections, were explored so that now the importance of this technique is no longer confined to disorders of the blood, but has become a valuable diagnostic aid in general medicine In many disturbances of hæmopoiesis, marrow biopsy also aids in prognosis and in the evaluation of therapeutic methods and it has contributed much to the elucidation of pathogenesis and classification of certain diseases. The advantages of sternal puncture in medical practice are already acknowledged by all hæmatologists and have led to its use not only in the smaller clinics but even in domiciliary practice The examination of marrow smears does not make histological examination superfluous. We always examine sections of the marrow in doubtful cases. The internal organs of all the cases coming to autopsy were examined histologically in order to compare the results of biopsy and of histological examination Our own histological sections were, with few exceptions, made at the Institute of Pathological Anatomy of the University of Berne under the direction of Professor Wegelin, to whom we wish to express our gratitude

The arrangement of the material examined and discussed in this book remains unchanged from the previous monograph, 1941, but the number of cases has now increased to more than 900, and many of them have been examined repeatedly As it is the aim of marrow biopsy to supplement rather than to replace older methods of investigation, in each case not only the blood picture but also the clinical findings and when available the result of autopsy are given

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CHAPTER II

TECHNIQUE OF STERNAL PUNCTURE

Choice of the Site of Puncture

WE use Arinkin's method of sternal puncture as it is applicable to infants as well as to adults and older children. Puncture of the tibia was practised previously on the incorrect assumption that it was impossible to obtain marrow from the sternum, particularly in infants The sternum is eminently suitable for marrow biopsy. Custer and Ahlfeldt (1932) found that sternal marrow becomes fatty only late in life (sequence of transformation: tibia, femur, ribs, sternum, vertebræ), and on the other hand activation of the marrow in regenerative processes occurs early (sequence: vertebræ, sternum, femur, tibia). The second or third intercostal space is usually chosen as the site for the puncture (Fig. 1), but when carcinoma is suspected the manubrium should be punctured as metastases are said to be more frequent there. In old people, when unsuccessful in the second or third interspace, the puncture should be repeated at the level of the fourth or fifth interspace. This site is suitable for routine puncture because Passler (1931) found that the transformation of red marrow into fatty marrow commences in the manubrium. Henning and Korth (1934) use this distal site for puncture regularly even in children, but we agree with Schulten (1937) that it cannot be recommended at that age owing to the elasticity of the lower part of the sternum and the possibility of the presence of cracks in the bone (Pässler) To avoid such cracks, the area chosen must be carefully palpated before the puncture. It should always be made at the level of an intercostal space, because cartilaginous areas may be met at the level of the joints between sternum and costal cartilages In adults at the junction of the manubrium and body of the sternum they have been [illegible] up to 90% of cases, but in the body it[illegible] are relati[illegible] mon.

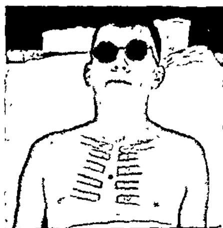


FIG 1 Site for sternal puncture

In cases where ma[illegible] se is s[illegible] e recommend with Henning (1935) t[illegible] cture sh[illegible] de at the site where metastases are [illegible] Spont[illegible] or X-ray

photographs, may help to localize a metastasis. We have punctured ribs, the greater trochanter, and vertebræ. The upper rim of the pelvis (Schulten, 1937), and the spines of the vertebræ (Heidenreich and Heidenreich, 1936) are easily punctured as we have confirmed, but in adults long bones need to be trephined An exception to this rule is the external malleolus which may be punctured, but will yield only scanty marrow particles, usually generously mixed with blood (Christen and Greif, 1938). Thus if sternal puncture fails, a variety of bones may be tried.

Instruments

Arinkin (1929) used a lumbar puncture needle and we used this until improved instruments were devised Grunke (1938) still recommends a short lumbar puncture needle, which he drives through the cortex of the bone with the aid of a wooden mallet. This method is not very satisfactory because it is important to feel the sudden lack of resistance, when the marrow cavity is reached. A large number of puncture needles have been suggested, but those with a guard first introduced by Arjeff (1931) are most useful We have used Rohr's cannula and the instrument suggested by Klima and Rosegger (1935), both of which have a useful guard, which in the latter model runs on a thread, and therefore cannot slip (Fig 2) A good sternal puncture needle must in addition incorporate the following features (1) it must not be too long, so that pressure on it does not produce a danger of bending or breaking, (2) its size should depend on the age of the patient In infants a thinner needle may be used but for adults we prefer a lumen of 1–2 mm to enable small marrow pieces to pass (3) The cannula must be fitted with a well-fitting trocar The bevel should be short so that the opening lies in

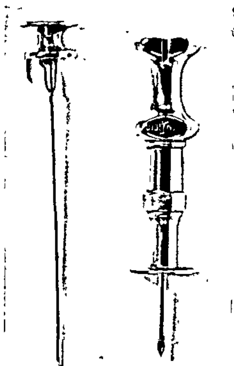


FIG 2 Sternal puncture needle. Klima's pattern, modified by Leitner

the marrow cavity even when the spongy bone is thin, and it should be sharp, to avoid slipping off the periosteum. Henning and Korth (1934) suggested a cannula with a side opening to facilitate irrigation of the marrow cavity. Their cannula was graduated in centimetres, which we believe is unnecessary, as the cortex is usually easily felt, and it had no guard so that there was no protection against accidents. These authors state that they can obtain marrow material by injecting 1 ml. of heparin or sodium citrate solution even when simple puncture has failed. Only exceptionally have we used sternal irrigation, and then without success in a case of fatty marrow; it may, however, be useful when the marrow clots quickly, as we have observed in uræmia, panmyelopathy and in the case of a cancer patient with leucoerythroblastic anæmia. Irrigation can be carried out equally well with an ordinary sternal puncture needle.

Additional instruments: a syringe, with a fine needle; 2 ml. of 0·5%–1·0% procain; a dry syringe with well-fitting plunger, for 2–10 ml., which must fit accurately into the puncture cannula; filter paper, slides (preferably thin ones), cover slips and a platinum loop.

Procedure

(Though Amprino and Penati (1935), Roversi and Tanturri (1935), Young and Osgood (1935), Mallarmé (1936) and also De Weerdt (1939) state that local anæsthesia is unnecessary, we think it preferable After raising a small bleb in the skin, the subcutaneous tissue and especially the periosteum are infiltrated, using 1–2 ml of 0 5%–1·0% procain. Within a few minutes of injecting the local anæsthetic the puncture may be carried out smoothly, without pain With poor analgesia or without any at all, the patient will feel the skin penetration and often becomes restless and this makes the later stages difficult. The patient is less likely to object to a repetition of the puncture if he has had no pain on the first occasion. As the penetration of the skin by the puncturing needle requires a certain amount of force, the point should be directed obliquely to avoid it going too deep Once the skin is perforated, the needle is then pushed vertically as far as the periosteum, while two fingers of the other hand fix the skin and subcutaneous tissue between two costal cartilages Once the periosteum is reached, the guard is fixed about 5 mm. above the skin level The cortical layer of the bone is always less than this, so that it can be penetrated, but at the same time the guard prevents the needle passing too deeply The puncture is made with a quiet, steady, rotatory movement while the patient holds his breath in full inspiration As the thickness of the cortex varies individually (Arjeff and Segerdahl give it as 0 5–1 0 mm) it is safer to remove the trocar and attempt gentle suction when in

doubt as to how far the needle has gone. Usually penetration of the cortex is felt distinctly as a sudden give, but we have often obtained marrow without having felt the passage of the cannula through the cortex, especially in children and young women. In old persons the cannula almost falls into the marrow cavity when the cortex is pierced. When the guard of the cannula is set at 5 mm. above the skin, there is as a rule no danger of penetrating the posterior cortex of the bone; as the thickness of the spongy bone usually amounts to 0·5–1·5 cm. (Arjeff, 1931). So far we have not had a single accident, but we always take the precautionary measures just described.

Breitenecker (1943) reported two deaths, and Alder (1944) one death, following sternal punctures inexpertly carried out. The sternum was completely penetrated and the right side of the heart injured. Inexperienced workers should adhere strictly to the suggested technique Hadorn (1944) reports fatal cases from shock in myeloid leukæmia, but such cases are probably inevitable even with very careful technique and Meyer and Halpern (1944) report a further death from shock and fear. The risks of sternal puncture have been reviewed in the *Lancet*, 1948, I, 566.

Whitby and Britton (1946) recommend that the sternal puncture needle be driven through the outer plate of the bone by *gentle* taps from a small hammer. They find that it is much more pleasant to the patient than the "pressing" method and is less likely to cause fracture of fragile bones. No accident has yet been reported using this "tapping" method.

We use a well-fitting 5–10 ml. syringe to obtain the marrow by suction, but a 2 ml syringe often suffices. When suction is made the patient experiences a short, rather sharp pain This may be lessened by slow withdrawal of the plunger. Arinkin used to aspirate about 10 ml. of marrow, but we withdraw only 0·1–0·3 ml, which takes a shorter time and is much less painful. In fact withdrawal of more than this quantity leads to an excessive admixture with blood and therefore disturbance of the differential count of the bone marrow cells and should be avoided

In our experience punctures without any yield are very rare. We have had two dry punctures (fatty marrow) Schulten (1937) and Thaddea (1943) report that in two-thirds of their cases they obtained marrow, Young and Osgood (1935) report 10% failures, which Klima (1938) explains by inadequate technique If at first suction fails to yield marrow, various manœuvres may lead to success. The cannula may be slightly withdrawn, then pushed forward a little way in a different direction By turning the needle and by repeated withdrawal and insertion of the trocar (Undritz, 1937; Stahel, 1938), the marrow may be stirred and then aspirated. If, after this technique, no marrow is obtained, an injection of 0 5–1 ml of sterile 3 8% sodium citrate solution at body temperature may produce some marrow fragments We do not, how-

over, approve of sternal irrigation as a routine procedure, particularly as failure of the puncture may be more apparent than real. We often find, especially in the case of tumours, that if the cannula is rinsed through, after withdrawal, small pieces of marrow containing tumour cells may be obtained.

The marrow fluid obtained is expelled into a watchglass, and the fragments of marrow which are usually easily seen are picked up with a platinum loop or with the edge of a slide. To get rid of the blood, Storti (1937) suggests blotting it up with filter paper. Vischer (1938) uses a piece of cloth We prefer filter paper, as it causes the loss of fewer fragments of marrow. A slight dilution with blood makes the preparation of films easier. We therefore take the small marrow pieces with a little blood from the watchglass. Piechl (1942) dips the marrow pieces into marrow blood before spreading films, but this seems unnecessary if some blood is left in the watchglass after removing the excess with filter paper Because marrow clots very quickly owing to its high fibrinogen content it is important to work speedily. Keilhack (1938) states that marrow contains three times as much fibrinogen as the blood. Gordon (1941) withdraws 1 ml. marrow, mixes it with oxalate powder and makes smears or histological sections from the mixture after centrifuging. Whitby and Britton (1946) withdraw 0·25 ml of marrow fluid and mix it with Heller and Paul's dry ammonium and potassium oxalate mixture in quantity just sufficient for making an isotonic suspension of the marrow They then make films on glass slides in the ordinary way, at the same time a total nucleated cell count can be carried out as described below Amprino and Penati (1935) use a sedimentation method with a mixture of 2–3 ml. marrow in Tyrode's solution To avoid admixture with blood Weller (1937) perforates the sternum obliquely at an angle of 45 degrees He then removes the trocar and pushes the needle a little further on He does not use suction, but examines only the small amount of material found in the lumen of the needle. In our hands this method was not very satisfactory, but we have often made use of the marrow sediment of a citrate mixture)

Preliminary Examination of the Material

Occasionally one fails completely to obtain marrow by sternal puncture, and sometimes only a little marrow is obtained with great
difficulty. Such difficult punctures may [illegible] osteosclerotic
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a case of osteosclerotic anæmia on the basis of hardness of the sternum and their diagnosis was later confirmed by X-ray. In the case of a female patient with hyperadrenalism and osteofibrosis, Leitner (1944) found difficulty in sternal puncture due to the extreme thickness of the cortex.

Examination of the material in the watchglass often gives valuable information for diagnosis. Macroscopical fattiness of the marrow is occasionally apparent since fatty droplets float on the surface, and marrow pieces, when rich in fat, do not sink, but float on the mixture. With a purely fatty marrow, puncture occasionally produces no material at all, but the trocar when withdrawn glistens with fat and thus suggests the cause of the failure. We have found fatty marrow in advanced age, after X-ray therapy and in certain infections, for example in the senile type of tuberculosis. Normally the colour of the marrow fragments is greyish-yellow; in leukæmia it is often grey. The pieces are relatively large in hyperplastic marrow. They are usually small and compact in young individuals, often bigger and flabby in older people. In untreated pernicious anæmia, the marrow is mostly red. In a case with carcinomatous deposits in the bones we have repeatedly found small blackish marrow fragments (Leitner, 1945).

Sources of Error in Sternal Puncture

Like almost all aids to clinical diagnosis, sternal puncture has certain limitations which must be realized. Dameshek, Henstell and Valentine (1937) compared Seyfarth's trephine method and Arinkin's puncture method in parallel examinations and observed certain drawbacks to the puncture method They found that puncture yields fewer primitive cells because the early forms adhere more firmly to the tissue stroma. The number of reticulocytes was found to be smaller in puncture than in trephine material. The trephine showed the proportion of red to white cells as 1 : 1, whereas in punctures it was 0.5 : 1 These differences probably depend on the variability with which cells may be dislodged from their attachments. Piechl (1942) examined this phenomenon in smears He made smears from the small fragments of marrow on a slide in a definite line and followed up this line microscopically He found erythroblasts more sticky than granuloblasts, a fact which we have confirmed Dameshek and his collaborators (1937) prefer the trephine in cases of tumours and in Gaucher's disease, because the cells are obtained with their tissue connections. Favorite (1939) suggests a method for obtaining pure marrow by combining puncture and trephine. A sharp trocar and cannula is pushed down to the sternum, the trocar is then withdrawn leaving the cannula in position and through this he introduces a borer, which may be screwed repeatedly into the marrow. The material

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Preliminary Examination of the Material

Occasionally one fails completely to obtain marrow by sternal puncture, and sometimes only a little marrow is obtained with great difficulty Such difficult punctures may be due to osteosclerotic processes or may occur with certain tumours, in some cases of chronic myeloid leukæmia, marrow aplasia, myelofibrosis and marble bone disease. An unusually easy puncture may suggest a very thin cortex due to osteoporosis (pernicious anæmia, decalcification, advanced age, etc.) as reported by Markoff (1936) and Leitner (1941) Weil and Perlès (1940) discuss the use of sternal puncture in assessing bone calcification. Binder and Riedl (1942) diagnosed

a case of osteosclerotic anæmia on the basis of hardness of the sternum and their diagnosis was later confirmed by X-ray. In the case of a female patient with hyperadrenalism and osteofibrosis, Leitner (1944) found difficulty in sternal puncture due to the extreme thickness of the cortex.

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collected in the grooves of the borer is then examined, admixture with blood being thus avoided.

Another source of error is the fact that bone marrow is not homogeneous In 32 bodies examined at autopsy Helpap (1937) examined the sternal marrow, and in 24 of these the femoral marrow. He found various differences in the cellular composition of both types of marrow. Reiter (1938) in a series of 100 cases found irregular, fairly large foci of fat in the sternum in 63 Twenty of these had small foci in the manubrium, 41 had them also in the body of the sternum, and 2 had them in the body only. Fatty patches are often found at the level of the second intercostal space, and these might have simulated marrow aplasia if punctured. The cellular composition of the active marrow was, however, identical in the individual parts Domarus (1937) illustrates the lack of uniformity of the marrow with cases. In a young woman suffering from anæmia, puncture showed regenerating marrow, but histological section showed a purely fatty marrow. Repeated punctures might conceivably have corrected such fallacies. It has already been stressed that a single puncture only shows a momentary picture in the same way as a single X-ray examination Repeated punctures will permit reliable interpretation in cases of doubt. Stasney and Higgins (1939) examined marrow from ribs, femoral epiphyses and diaphyses They found the cellular composition in the various sites fluctuated within narrow limits, and therefore did not consider that the source of error from the lack of uniformity of the marrow was great Jeanneret (1940) found appreciable differences between sternal and costal marrow, but we have not been able to confirm this. In 10 cases we punctured sternum, ribs and occasionally also vertebræ immediately after death and observed uniformity in cellular composition We have not, however, examined the long bones In hyperplasia of the bone marrow the cellular composition appears uniform, but in hypoplasia several punctures in different areas may be necessary to get a correct picture

Sources of error undoubtedly exist and must be kept in mind They may be much reduced by the use of the methods mentioned above.

Repeated punctures do not influence hæmopoiesis (Stodtmeister and Buchmann, 1939), and therefore repeated examinations do not introduce new sources of error, but rather reduce them

Further Technique; Staining

It is necessary to count at least 300–500 cells in a few smears before a moderately reliable verdict can be given Schulten (1937) suggests omitting a differential count altogether, because the physician usually has not sufficient time to examine the preparation

personally and so this work becomes the duty of laboratory assistants. He prefers a thorough general search of the preparation by the physician or pathologist, and Thaddea (1943) agrees with this view. Schulten's argument is well worth considering, because a proper examination of the bone marrow, including a differential myelogram, an oxidase preparation and a supravital preparation with staining and total count of marrow cells takes several hours to complete. In spite of this warning we prefer and advocate a differential count of the myelogram, partly because of accuracy and comparability of the results, and partly because the examiner is thus forced to make up his mind about each individual cell and becomes more and more certain in the definition of the various cell types. We carry out a differential count in all our own preparations, after a thorough general search as Schulten suggests For routine purposes a total cell count, a differential myelogram and possibly a supravital preparation are sufficient, but in certain cases other investigations become necessary and these will be briefly discussed.

For the total marrow cell count we use an ordinary white cell pipette, and draw marrow fluid up to the mark 0·5 on the capillary stem. We dilute up to mark 11 with the usual white cell diluting fluid (glacial acetic acid 3·0, 1% watery gentian violet 3 0, distilled water ad 300·0) The total count is carried out in a counting chamber of the Schilling or Neubauer patterns, counting the nucleated cells in the four corner squares, and in the fine marked centre square, just as in a total leucocyte count.

The smears are made by carefully drawing apart two coverslips or slides, after having placed one to two marrow pieces with a little blood on one slide In order to avoid squashing the cells, pressure on the slides must be avoided. The smears are allowed to dry in air for 12–20 hours

For Staining Permanent Preparations. The May-Grunwald-Giemsa method is recommended. The slide is flooded with Jenner-May-Grunwald solution and left for 2 minutes Distilled water is added and left for 1 minute To avoid precipitation of the stain, it is preferable to stain the slide inverted in a special dish, but even with the ordinary method precipitation is not common After rinsing the slide with distilled water the film is stained with Giemsa stain The solution must be prepared freshly every time Various dilutions are used We add 8 drops of stain to 5 ml. distilled water and leave the mixture on the slide for 12–15 minutes, depending on the thickness of the smear The slide is then rinsed in distilled water, after which it should be examined with a dry lens of the microscope in order to see if nuclear staining is adequate The slides are then stood vertically and allowed to dry. We do not like blotting with filter paper, and only use it when an examination is very urgent This combined method usually produces very fine preparations The May Grunwald solution stains the cytoplasm

and Giemsa stains the nuclei distinctly so that the structural details are easily recognizable with the $\frac{1}{12}$-inch oil immersion lens.

Instead of this panoptic method, after fixation with methyl alcohol an ordinary Giemsa stain (without May-Grunwald) may be used. *This method, too, produces beautiful preparations, but it does not stain the granules of the mast cells, as they are dissolved* by methyl alcohol. This helps to distinguish the ordinary basophil granulocytes and their precursors from the tissue mast cells, as the thick granules of the latter are stained by the ordinary Giemsa method. Such differentiation is also possible with the panoptic method, because the tissue mast cells have small, round, dark nuclei (rich in chromatin) and very thick, intensely black-purple granules (see Figs. *165 and 166, p 267), while the mature* blood mast cells have a lobed and the juvenile basophil myelocytes a round, longer and lighter nucleus, with only scanty purple granules (see Fig *14, p 29)* *These appearances led Undritz* to speak of basophil cells with soluble granules and those with insoluble ones

Whitby and Britton (1946) recommend simple staining with Leishman's stain, the length of time of staining depending on the cellularity of the marrow smear.

We also make a supravital preparation as soon as the ordinary smears have been prepared. Our own method (Leitner, 1935) *follows those of Schilling (1933) and* Heilmeyer *(1942).* Slides are prepared by placing at one end a drop of 1% alcoholic brilliant cresyl blue solution, which dries as a coloured ring on the slide *A marrow fragment, together with a small drop of marrow blood,* is placed on this coloured ring and mixed thoroughly with the stain by manipulating the spreader for 1 minute The mixture is then spread like an ordinary film The still moist preparation is placed for 10–15 minutes in a moist chamber (*e g*, Petri dish containing a piece of moist cotton wool) In supravital preparations all the cells are stained light blue, the cytoplasm relatively deeper than the nuclei, the eosinophil granules are coarse and intensely blue and the platelets are light blue. The reticulocytes for which this method was originally described are well defined Their reticulum appears as a dark blue network or as a granulation on a slightly greenish *background of cytoplasm* The nuclei of erythroblasts are deep blue, often surrounded by a reticulum. We examine all marrows by this supravital method, and have gained valuable information *about the mechanism of the loss of nuclei from the* erythroblasts. Klima (1938) prepares his slides with 0·5% alcoholic Nile blue sulphate before making the supravital preparation The nuclei of cells and *the platelets appear blue and fat droplets* brownish-red, so that this method is suitable for a study of cells which phagocytose fat We have used a supravital method with alcoholic Sudan III for this purpose

Peroxidase Staining. We frequently use peroxidase staining methods. For routine purposes we found Moschkowski's method most suitable It produces uniformly good preparations and, when counterstained with Giemsa solution, the preparation can be compared with ordinary marrow smears. The dry marrow film is fixed for 3 minutes in 90% alcohol and after a rinse in water is covered with freshly prepared benzidine-perhydrol solution for 5–10 minutes. The slide is rinsed in distilled water and as usual counterstained with Giemsa solution for 12–15 minutes The benzidine-perhydrol solution is prepared by dissolving a few crystals of benzidine in warm water and adding ½–1 drop of 1% hydrogen peroxide solution per ml of benzidine solution Oxidase positive cells show golden-yellow or yellow-brown granulation, which is so dense in neutrophils and their precursors that the cytoplasm appears like a compact brown mass (Fig. 194, p 413). Monocytes usually show scanty, but on the whole easily recognizable, yellowish granules Eosinophils have glistening, round, light-brown, deeply-stained granules. Mast cells as well as lymphocytes remain negative

We have also used Graham's peroxidase method, but it is a little more complicated. The film is fixed with a solution containing 90 ml. of 95% alcohol and 10 ml. of 40% formol; rinse, flood with fresh benzidine-perhydrol solution consisting of a few benzidine crystals, 0 02 ml. hydrogen peroxide and 10 ml. of 40% alcohol for 5 minutes, rinse again and counterstain with watery anilinthionin (10 ml. saturated solution in 75% alcohol made up to 40 ml anilin water).

Sato's peroxidase method is widely used Dry films are covered for 30 seconds with a 0·5% copper sulphate solution, then for 2 minutes with a benzidine-perhydrol solution (benzidine 0 2 grams, 4 drops hydrogen peroxide and 20 ml distilled water) After rinsing in distilled water counterstain with 1% safranin for 2 minutes The blue oxidase granules contrast very well with the red stained nuclei (Fig 171, p 298) The relatively frequent precipitation of copper sulphate crystals is a disadvantage of this method Differential counting is not so easy as in Moschowski's method

Unna-Pappenheim's method with methylene green and pyronin is a very simple one and has proved its value for the recognition of lymphocytes and their precursors and plasma cells After fixing with heat or alcohol the slide is covered for 5 minutes with carbolmethylene green-pyronin solution, then rinsed and blotted. Pyronin stains the basophilic cytoplasm of lymphocytes and plasma cells intensely red, while the nuclei are bluish-green. In glandular fever, infective hepatitis and other conditions this method is useful for the identification of lymphoid cells and for their differentiation from monocytes

Sabin's (1923) supravital preparation with neutral red and Janus

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CHAPTER III

CELL COUNTS ON THE STERNAL MARROW

THE total number of nucleated cells per cmm. is not counted by all authors, since Segerdahl (1935) found variations between 7,000 and 100,000 We always prefer, however, to make a total count of the marrow cells, in a Neubauer or Schilling counting chamber, as the total number of cells per cmm is of value in aplastic marrow reactions The normal number of cells in our experience is 60,000–100,000 per cmm which, although a fairly wide range, is within smaller limits than Segerdahl's figures Greif (1937, 1938) found a margin of error of ± 6·54% in total nucleated cell counts. It is of first importance to remember that if an accurate nucleated cell count is to be carried out a constant quantity of marrow fluid should be withdrawn. It has been shown that if more than 0 3 ml of marrow fluid is aspirated admixture with blood is unavoidable and the greater the amount of fluid withdrawn the greater the error.

There are a number of methods of making a differential count of marrow cells Schilling (1925) counts all nucleated cells and records the number of nucleated red cells per 100 of the other nucleated cells Segerdahl (1935), Grunke (1938) and Whitby and Britton (1946) express the various types of nucleated cells in percentages, but Rohr (1937) gives the white cells only in per cent. and then records the number of nucleated red cells and of reticulum cells per 100 white cells We construct our myelograms according to Schilling's principles. The results of various authors are summarized in Table 1 Our own figures are average values obtained by twenty-two sternal punctures on healthy subjects Experience gained since our previous papers has confirmed the accuracy of these results.

The figures vary considerably, but Table 1 also includes rather earlier results It is worth mentioning that many authors arrived at their average values on the basis of few examinations only, sometimes only 4–10 cases, so that any abnormal count in such a small series makes a considerable difference Figures obtained during the last few years, however, agree sufficiently to supply a basis for practical work Great variations were also met with in results from trephining of the sternum. According to Schilling (1925) the numbers of myelocytes vary between 35 and 47%, according to Barta (1933) between 40% and 42% according to Dameshek (1935) between 15% and 25%, according to Arinkin (1929) between 4·5% and 8 6%, and according to Whitby and Britton (1946) between 2%–8% Dameshek quotes the number of erythroblasts as 50%, Schilling 31%–44%, Barta 30%–33%, Arinkin 5 7%–16%, Weiner and

TABLE
Normal myelograms recorded

Cells	Arinkin (1929)	Escudero and Varela (1932)	Holmes and Broun (1933)	Young and Osgood (1935)	Tempka and Braun (1932)	Nordenson (1935)	Segerdahl (1935)	Rohr (1940)
Leucopoiesis :								
Myeloblasts	1–2·4	5·5	2·4	0–1·2	4·7–7	0·25–5·5	1·3	1·3
Promyelocytes	1–2·8	9·0	—	0–7·8	3·75–8·8	1·25–8·2	1·4	9·5
Neutrophil semi-mature myelocytes	4·5–8·6	20·5	7·0	0·2–6	12·7	4·25–18	16·0	2–17
Neutrophil mature myelocytes	4·5–8·6	20·5	7·0					
Neutrophil metamyelocytes	1·4–3·4	27·43	6·7	1·8–9	14·3	12·5–42·0	15·7	3–11
Neutrophil stab cells			14·0	15·8–33	17–22	2·52–10·0	10·0	35–50
Neutrophil segmented polymorphs	41·0–55		17·4	7·4–25·2	16–20	14·25–35·0	21·0	11–29
Eosinophil myelocytes	0·3–1	0·9		0–0·4	1·5–2·6			
Eosinophil metamyelocytes	0·3–1	0·73		0–2	0·3–3·6	0·25–7·5	1·4	2–7
Eosinophil polymorphs	0·6–4		1·0	0–1	0·6–2·5			
Basophil myelocytes		0·2			0·13	0–0·75		
Basophils	0·1–0·7			0·06				
Lymphocytes	7·3–16·5		24·9	4·8–16	2·6–3·2	7·5–38·0	17·0	2–19
Monocytes	2·1–9·3		9·0	0–4·2	0·5–0·7	0–5	2·0	1·5
Megakaryocytes	0·6–6·1			0–0·2	2·1–4	0·1	0·03	
Endothelial cells		2·23						
Plasma cells	0·3–0·9			0–1				
Hæmohistioblasts		2·23			1·33–3	3–4		
Phagocytic reticulum cells		9·86						
Lymphoid reticulum cells							0·03	7·0
Erythropoiesis :								
Proerythroblasts	0·8–2·9	5·5	5·2			0–6		4·4
Early normoblasts	} 5·7–16			5·4–20		1–16	} 12·88	9·3
Late normoblasts		13·0	6·9			26·0		16·4
Unidentified cells				12·8–31·8	0–3	0–4·5		

Kaznelson (1926) 19·4%, Rohr (1937) 30·1%, Greif (1938) 25·92%, Napier and Sen Gupta (1938) 32·2%, de Weerdt (1939) 45·02%. The number of unidentified cells becomes smaller with increasing experience. Schilling found 11·6%–24·8%, Schulten (1937) up to 20%, and we formerly found 3·5% of such cells, but with continued experience we believe that this figure is too high and that the unidentified cells should not be more than 2%. In 22 normal cases we found the following figures for the degree of segmentation among the neutrophils. These are the first details so far published.

TABLE 2

Stab cells per 100 neutrophils	52
Cells with 2 segments	32
Cells with 3 segments	14
Cells with 4 segments	2
Cells with 5 segments	0

1

by various authors

Mallarmé (1937)	Markoff (1936)	Klima (1938)	Fleschl (1940)	Révol (1938)	Weil and Perlès (1938)	DeWeerdt (1939)	Leitner (1941)	Henning and Keilhack (1939)	Greif (1937)	Thaddea (1943)	Whitby and Britton (1946)
2·5	1·5	1·0	1·52	0·75–3·5	0·5–1·5	1·76	1·2	0·6	1·55	4·0	0–2·5
2·5	1·5	1·0	1·52	0·75–3·5	0·5–1·5	1·76	2·2	0·6	1·55	4·0	0·5–5
1·5	3·0	3·0	2·98	2·9–7	1·0–2·0	4·67	5·4	2·4	2·62	2·62	
17·5	12·0	14·0	12·4	10–12	30–35	20·76	7·2	7·9	11·32	15·0	2–8
12·0	12·5	14·0	2·2	0·5–2	10–15	23·9	10·2	6·3	12·06	4·0	2·5–12
	13·0	11·0	13·2		24–30		24·0	31·3		10·0	
32·5	31·0	18·0	17·64	10–35		24·92	28·4	8·2		15·0	20–50
2·5	1·5	1·0	2·2	0·5–2		2·27	1·4	1·6	1·23	1·0	0–1
0·5	0·4	0·5		0·5–2		0·79	0·8	0·8	0·03	1·0	0·2–5
2·0	3·0	0·7	2·4	0–0·25	0·5		1·8	0·7		1·0	0–4
	0·05						0·02	0·2		0·5	
0·04	0·5						0·02	0·1			0–1
9·5	15·0	7·0	4·18	6–15	14–21	7·32	7·6	2·7	17·84	10·0	3–20
2·5	1·5	1·0	2·44	2–6	2·3	3·3	1·4	0·7	1·7	2·0	0–5
0·06	0·05			0·1–0·5		0·4	0·8	0·1	0·47	0·5	
	1·0						0·8				
0·9			0·96	1–4	0·5–1	2·16	1·0	0·1	1·11	1·0	0–1
		0·5		0·25–3·0	0·2–0·5	0·75		0·3			0–1
			1·78	5–25			0·2	1·6		0·5	
							1·0				
6·0	0·01	1·5	3·56	0·5–15	1·0	0·65	0·8	1·8			0–4
6·0	2·5	7·0	11·5	} 10–38	} 15–20	3·22	3·2	6·4	} 25·92	} 23·0	4–15
10·0	10·55	18·0	9·4			41·15	24·4	21·5			7–19
			2·9				0·35	0·7			

The degree of segmentation is important in practice in pernicious anæmia and in familial anomalies of leucocytes (Pelger-Huet anomaly) Escudero and Varela (1932) and also Pontoni (1936) exclude mature granulocytes in counts of the marrow cells because *they believe that their presence is due to admixture with blood.* That this is not true is shown by the fact that we were able to demonstrate mature granulocytes in appreciable numbers in the marrow in cases of agranulocytosis where they were completely absent from the peripheral blood An interesting case from this point of view, the sternal marrow of which we examined, has recently been published by Tobler and Buser-Pluss (1942). Since in the blood differential count also, considerable variations are taken as normal (for example, monocytes 1%–7%) without casting doubt on the value of the hæmogram, we believe, in spite of all criticism, that the figures counted for myelograms are of practical value

The subdivision of individual species of cells (megakaryoblasts, plasmoblasts, etc.) *will be considered later*, but a few remarks seem necessary about the changes in the cell count according to the age of the patient. Markoff (1936), Barasciutti (1937), Reiter (1938), Seggel and Reiher (1939) and Leitner (1941) noted that the marrow became *poorer in cells and more fatty with advancing age*. Videbaek (1941) tried to elucidate this problem by a series of 236 sternal punctures on persons between seven and seventy-two years. He found the total number of mature cells higher in children than in adults, *but after the fifteenth year the* marrow composition remained constant. Our results agree with this. The numbers of plasma cells and of reticulum cells were low in childhood (1%) and increased with age to 3%. The numbers of erythroblasts increased slightly with age. The differences, however, were not great enough to lead to errors in diagnosis. Shapiro and Bassen (1941) noted in babies during the first few weeks of life an increase of myelocytes from 61% to 77%, and a decrease of erythroblasts from 32% to 12%. They believe that neonatal anæmia is not due to hæmolysis, but to a reduced production of erythroblasts. According to Conti (1941) erythroblasts dominate the picture in the first week of life, but soon decrease. Jacobsen (1941) examined 88 cases and found in children more primitive white cells and lymphocytes (especially in children under five), but fewer erythroblasts than in adults. There were apparently no differences between younger and older adults.

Apart from percentage figures, many ratios and curves have been established. Picena's (1937) *karyokinetic* index is based on the number of mitoses per 100 cells of the same series. This amounts to 0·1–1 for the granulocyte series and 2·72 (1–4·6) for erythroblasts. Fieschi (1938) attaches great practical importance to karyological curves. *These are the plotted percentage figures of the individual* stages of mitosis and will be discussed later. De Weerdt (1939) determined a maturation index, which is the ratio of proerythroblasts and basophilic erythroblasts to mature normoblasts. This index, *according to our own figures*, is normally 6·5. The proportion of nucleated red cells to white cells is estimated by Picena (1937) as 1 : 3·08, by Rohr (1937) as 1 : 3·28. Our own findings give the ratio as 1 : 3·52, so that roughly a ratio of 1 : 3 may be considered normal. The maturation curves of erythroblasts (Pontoni, 1936) and of granulocytes do not appear any more useful than the percentage figures of the different stages in development though the plotted curves are very instructive for rapid surveys. Other indices have been established by Bock (1939), Cotti *et al* (1938) and others.

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CHAPTER IV

MORPHOLOGY OF THE MARROW CELLS

THE discrepancies between the different figures given by various authors in the myelogram (Table I, p. 18) are partly due to differences in classification of the marrow cells. Morphological studies have been much advanced since the days when the examinations were carried out chiefly on autopsy material. Marrow cells undergo rapid degenerative changes post-mortem, owing to agonal and post-mortem acidosis, as shown by Schilling and collaborators (1933), Rohr and Hafter (1937), Jeanneret (1940) and our own observations The changes are not very marked for the first hour after death, but 8–24 hours post-mortem the number of mature granulocytes falls to 0·35% Myelocytes undergo morphological changes, become vacuolated and are then less easily recognizable. Erythroblasts often show karyorrhexis. The reticulum cells are the most resistant to post-mortem changes, and plasma cells are still easily distinguishable 20 hours after death Rohr and Hafter believe that sternal punctures are of doubtful value 5 hours after death, while Jeanneret considers 1–3 hours after death the time limit for laboratory examination. Our own observations have convinced us that sternal puncture half an hour after death is already too late to give a result of any value Post-mortem changes occur more quickly in the presence of septic processes, and are delayed in agranulocytosis and in multiple myeloma Nineteen hours after death only cells with round nuclei are seen in the marrow (Figs. 4–5) We have observed that cytoplasmic changes precede nuclear changes, the cellular periphery becomes hazy, the non-granular basophil cells show a dull blue or grey cytoplasm, while the granular cells show increased granulation It is therefore clear that the finer morphological details are easier to recognize in life than after death

With regard to the formation of the different marrow cells, there are three main opposing theories, viz, the unitarian theory of Möllendorf (1926), Maximow (1932), Stockinger (1933) and others, the dualistic theory of Naegeli (1931) and Schulten (1939) and the trialistic theory of Schilling The polyphyletic theory, too, with its assumption of individual stem cells for each cell species, has its followers, *e g*, Undritz (1937). We believe that blood and marrow cells take their origin from the marrow reticulum cell If we trace the development of the cells as far back as that we come to the conclusion that undoubtedly there is much to be said for the unitarian theory The reticular stem cell is called the hæmohistioblast by Ferrata and the polyblast by Möllendorf We believe that the myeloblast first described by Naegeli, is the earliest morpho-

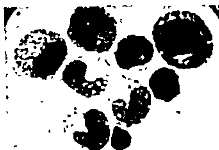


Fig 3 Post mortem changes Marrow obtained immediately after death Cell grouping still almost normal, but the coarsening of granules and haziness of periphery of cytoplasm is beginning. (× 1,050)



Fig 4 Post-mortem changes 3 hours after death decrease of numbers of mature granulocytes, coarsening of granulation, haziness of cytoplasm (× 1,050)

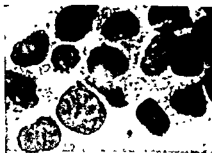
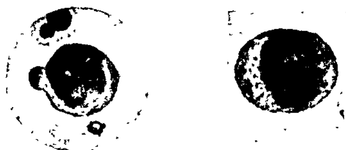


Fig 5 Post mortem changes after 24 hours only cells with round nuclei remain, cytoplasm almost dissolved (× 1,050)

logically recognizable precursor of the leucocytes, and corresponds to Ferrata's hæmocytoblast, and we consider the proerythroblast (Naegeli, Schulten and others) to be the earliest precursor of the erythrocytes Rohr (1940) maintains that after birth all hæmopoiesis is homoplastic, *i.e*, the development is from the precursors, of the same cell species.

The Red Cell Series

The proerythroblasts are called erythrogonia by Helly (1910), Ellermann (1923) and Askanazy (1927), but Schilling (1933) and later on Henning (1938) called still more primitive cells proerythroblasts, and they consider these primitive cells to be stem cells of the cells which we now name proerythroblasts and megaloblasts (Dameshek, Henning). Komiya (1938) thinks promegaloblasts and erythrogonia are identical We believe it is undesirable to use the same terminology for the stem cells of the pathological megaloblastic developmental series, as for the normal erythroblastic



Figs. 6 and 7 Proerythroblasts of varying maturity with nucleoli and basophilic cytoplasm, near one of them is a normoblast in karyorrhexis. (× 1,000) and (× 1,400)

series It is quite sufficient to consider the proerythroblast as a stem cell with both primitive and more mature forms. The same applies to each stage of development and the differences due to maturation similarly do not require a distinctive name The proerythroblasts have a primitive looking, finely reticular nuclear structure and a thin rim of strongly basophilic cytoplasm In the immature forms, which are usually bigger, the cytoplasm is at times more plentiful. Their size is 15–20 microns The round nucleus contains 1–2 nucleoli Proerythroblasts do not contain hæmoglobin

The next maturation stage of the normal red cell series is the early basophilic normoblast. It is a big cell, though smaller than the proerythroblast, with round coarsely reticulated nucleus and uniformly basophilic cytoplasm

Schilling (1933), as well as Dameshek (1935), designate by the name "erythroblast" the immediate precursors of the normoblast,

but we prefer to use this term as a collective denomination for all nucleated red cells.

The late normoblast is the most mature form of the erythroblast and shows a nucleus which becomes less spongy and more coarsely reticular depending on its maturity, till finally it becomes pyknotic. Its cytoplasm is polychromatic or orthochromatic.

We agree with Maximow (1932), Piccna (1937), Fieschi (1938) Klima (1938), Schulten (1939), and other authors that the staining reaction of the cytoplasm is a very good indication of the age of an erythroblast. Maturation curves of normoblasts have been plotted according to the staining reaction of the cytoplasm. Piccna found orthochromatic normoblasts most numerous; in our experience, however, polychromatic normoblasts predominate by far. De



FIG. 8 Early basophilic normoblast (× 1,400)



FIG 9 Polychromatic and orthochromatic normoblasts and a free pyknotic nucleus from a normoblast. (× 1,400)

Weerdt (1939) also found the orthochromatic variety only relatively rarely His figures were of 100 erythroblasts, 5 were basophilic, 75 polychromatic and 20 orthochromatic. We define the age of the erythroblasts on the basis of four criteria (1) nuclear structure, (2) cell size, (3) quantitative relationship of nucleus and cytoplasm, (4) staining reaction of cytoplasm

The next stage of development of the red cell series is represented by a reticulum which can be stained by supravital methods (reticulocytes) These have been subdivided according to the arrangement of the reticulum matter. ball of wool form, big and *small net forms and granular form* Numerous *supravital staining* methods are used to demonstrate them We employ a simple method (p 12), using brilliant cresylblue which enables both reticulocytes and platelets to be counted in one and the same preparation

We separate the pathological developmental series of megaloblasts from normal erythropoiesis Their precursors, promegaloblasts, are characterized by a fine chromatin structure, nucleoli and cell size (15–30 microns) With Naegeli (1931), Rohr (1937) and Whitby and Britton (1946) we believe that they are easily distinguished from early normoblasts, but Segerdahl (1935), Nordenson (1936) and Jagić and Klima (1937) find differentiation uncertain

The fact that certain authors, such as Komiya (1938), report the presence of promegaloblasts in healthy subjects, is due to mistaken definition. The next developmental stage of the megaloblast, which is so typical of embryonic hæmopoiesis and of pernicious anæmia, has been well recognized by all workers. This cell is easily recognized by the fine chromatin meshwork of the nuclei without the presence of any nucleoli. The broad cytoplasmic rim already shows perceptible hæmoglobinization and the nucleus is often oval, roundish or bean shaped (for illustrations, see Pernicious Anæmia, p. 72). Bock and Malamos (1939) compare the nucleus with a raindrop in dry sand. The early hæmoglobinization, while the nucleus is still finely reticulated, is characteristic, and provides evidence of the dissociation of nuclear and cytoplasmic maturation. The differentiation from proerythroblasts is made possible by the orthochromatic, polychromatic or weakly basophilic cytoplasm and by the absence of nucleoli; the fine nuclear structure differentiates them from the early basophilic normoblasts. While German, Scandinavian, Italian authors and others state that megaloblasts only occur in pathological (pernicious) marrows, some English, American and French writers (Sabin, Doan, Vogel, Napier, Weil) have suggested they are primitive forms in normal erythropoiesis, and this conflict has led to a certain amount of confusion about the definition of the megaloblast. We believe in the independent position of the megaloblast and consider that the wrong identification of early normoblasts and of proerythroblasts has led to this confusion Whitby and Britton (1946) agree with the independent position of the megaloblast series, but point out that Ehrlich's primitive megaloblast as found in the embryo is morphologically similar to the pathological megaloblasts of pernicious anæmia

Finally, it may be stated that marrow biopsy has given new impetus to the study of the loss of nuclei of the erythroblasts and the maturation of erythrocytes Schilling (1933) and Voit and Daiser (1936) believe that the nucleus is extruded in an intact form, Jolly (1904) suggests that the nucleus is extruded only after disintegration by karyorrhexis, Révol (1938) believes in extrusion of pyknotic nuclei Naegeli (1931) considers that the nucleus is dissolved by karyorrhexis and therefore disappears Whitby and Britton (1946) believe that all these processes play their part. Marrow biopsies favour Naegeli's view as with supravital staining all types of [illegible]

nuclei very rarely Boström (1944) postulates that erythrocytes form in the following fashion small *normoblasts* which remain [illegible]

from a state of gel to a *state of sol* and obtain a thin capsule [illegible] become completely detached from the erythroblast and then pass

into the circulation. Therefore several erythrocytes develop from one erythroblast, and this theory would also explain the migration of the erythrocytes from the closed vascular system of the marrow (Drinker *et al.*, 1922; Sabin, 1928; Doan, 1932; Rohr, 1940) into the blood stream. Schultz and Buding (1940) have previously advocated a similar theory for the origin of poikilocytes. From their studies on the formation of erythrocytes in rat embryos, Jacobsen and Plum (1941) believe that the cells develop simultaneously with the maturation of the nucleus as a sort of vesicle near the nucleus, which become detached. Afterwards the free nucleus perishes. Thus one erythroblast only produces one erythrocyte. It is, however, questionable if the assumption of a similar development of erythrocytes in man is justified. According to Habelmann (1940) extrusion of nuclei does not occur, but nuclear disintegration and nuclear dissolution do and they are real proteolytic processes. Basing his conclusions on marrow biopsies, he states that in rapid demands for cell repair nuclear disintegration predominates, but given adequate time karyolysis appears to prevail. We have found lysis to be more common even in conditions of great and rapid demand, *e.g.*, pernicious anæmia and the hæmorrhagic states. Only in toxic conditions or anæmia of malignant disease, etc., have we noted increased nuclear disintegration.

The White Cell Series

The evolution of the leucocytes is still a matter for discussion. Sternal puncture reveals the interesting fact that about 70% of the marrow cells belong to the white series, and only 30% to the red, whereas in the blood only two white cells per 1,000 red cells are seen. This indicates a shorter span of life for the white cells, and possibly also their slower maturation rate.

The myeloblast is the stem cell of all granulocytes, and according to the dualists (Naegeli, 1931, Jagić and Klima, 1937), also of monocytes and megakaryocytes, but the trialists (Schilling, 1933; Whitby and Britton, 1946) hold the view that the monocytes develop from the reticulum without the intermediate state of the myeloblast. We believe that monocytes are derived from the extramedullary and medullary reticulo-endothelial system and that megakaryocytes develop directly from the marrow reticulum cells rather than from the myeloblasts. Undritz (1937) goes one step further and presumes an independent stem cell for each species of granulocytes.

Myeloblasts are big cells with primitive, finely reticulated nuclei, several nucleoli, usually four, and cytoplasm of varying amount and without granules (Fig. 10).

The earlier forms are rather difficult to identify because they have a deep blue cytoplasm and may closely resemble procrythro-

blasts. These cells are often regarded as precursors of myeloblasts and proerythroblasts. The oxidase reaction does not help in differentiation because these very early forms are oxidase negative Hirschfeld (1932) states that only those cells which show at least traces of granules by the Romanowsky stains give a positive oxidase reaction. We have, however, found a positive oxidase reaction in completely agranular myeloblasts



FIG 10 Two myeloblasts with loose nuclear structure and three nucleoli each, and two neutrophil stab cells (× 1,400)

Myeloblasts are distinguished from proerythroblasts by their finely meshed nuclear structure, by their size, by the number and definition of nucleoli (they have four instead of one or two), and by the rather weaker basophilic reaction of the cytoplasm, and finally by other features which are not always distinct in individual cases In pathological conditions smaller forms also occur, termed micromyeloblasts by Naegeli, which often show pale cytoplasm Their differentiation from very early lymphatic cells is only possible by their rather looser chromatin structure, their nucleoli, the reaction of the cytoplasm and by mitotic figures Micromyeloblasts are very primitive cells, and are only met with in acute myeloid leukæmia, and it would perhaps be better to call them "promyeloblasts" or "proleucoblasts" Further pathological forms, the paramyeloblasts, which have an indented or even a lobed nucleus, will be discussed in the section on myeloid leukæmia

Promyelocytes represent the next developmental stage, and are easily recognizable. They are large cells, whose nucleus has retained a loose chromatin structure and nucleoli Their cytoplasm is basophilic, stains slightly lighter than in myeloblasts, but is broader and not homogeneous, and contains coarse spiky azure granules With Schilling (1933), Schulten (1939) and other writers we believe that the definition of promyelocytes should coincide accurately with the appearance of granules. The neutrophil semi-mature myelocyte is a little smaller than the promyelocyte and the nucleus-cytoplasm ratio alters in favour of the latter, this phenomenon being generally the rule in the advancing maturation of the neutrophils The structure of the nuclear chromatin is less fine, and nucleoli are usually absent The cytoplasm is less basophilic and, in addition to the azurophil granules, neutrophil granules become recognizable The mature myelocyte has a round nucleus with compact chromatin structure, the cytoplasm is neutrophil (as far as neutrophilia can be considered a permissible term, *cf.* Leitner and Eichhorn, 1932), and the granulations are very fine (Fig. 11). The nucleus is smaller

in relation to the cytoplasm and may show slight indentation as a sign of transition to the metamyelocyte.

The metamyelocyte has a fully mature cytoplasm (Fig 12).



FIG 11. Immature and mature neutrophil myelocyte (× 1,400)



FIG. 12 Two neutrophil metamyelocytes (× 1,400)

Pappenheim (1911), who coined the term metamyelocyte, defined it as an intermediate stage between fully mature leucocytes and myelocytes, and also included segmented cells with juvenile nuclear structure. To Schilling (1933) credit must be given for establishing a definition useful in practice by his description of stab cells and juvenile forms. These are unsegmented cells with distorted or indented nuclei, the structure of which is more mature in the stab cells, as shown by the compactness of the chromatin Rohr (1940) believes that promyelocytes develop into strongly segmented neutrophils, myelocytes into rather less segmented ones, and metamyelocytes into mature stab cells, but we cannot share this view The most mature neutrophils are the segmented ones, with more or less subdivided nucleus.

The eosinophil myelocytes can only be distinguished from the



FIG 13 Young, eosinophil myelocytes ("eosinophil promyelocyte") with multi coloured, partly blue and grey, and partly red granules (× 1,400)



FIG 14 Young, basophil myelocytes ("basophil promyelocyte") with finely reticular nucleus, including a big nucleolus, and coarse, dense granules, scattered over the nucleus (× 1,400)

neutrophils by their granules. Apart from the well-known coarse oxyphil granules, immature eosinophil myelocytes show blue or blue-grey granules, usually called "multi-coloured granulation"

(Fig. 13) With increasing maturity the blue and grey granules disappear and give way to a homogeneous red granulation. Metamyelocytes, stab cells and segmented polymorphs apart from the eosinophil granules, correspond to those in the neutrophil series, but the nuclei of the eosinophils are rather less segmented, and their chromatin is less compact.

Most authors, except Mallarmé (1937), divide the basophils into two groups only, myelocytes and mature mast cells. The granules are deep purple, rather smaller than those of the eosinophil, and appear to become lost quite easily during staining. The cells therefore often appear to be markedly vacuolated (Fig. 14). Mature basophil cells also have a nucleus somewhat poorer in chromatin than neutrophil cells.

The Monocyte and Lymphocyte Series

Opinions vary widely about the nature and origin of monocytes. Maximow (1932), Downey (1938) and others, following the unitarian theory, presume that monocytes are derived from lymphoid marrow cells. The dualists, including Ehrlich, Naegeli, Rohr and Schulten, trace them back to the myeloblasts. Primitive forms, called monoblasts by Forkner (1932) and Seemann (1930), may occur between myeloblasts and monocytes, but Hittmair (1942) denies the existence of monoblasts Thaddea and Bakalos (1939), basing their opinion on observations on a single case of monocytic leukæmia, believe that myeloblasts and promyelocytes are the stem cells of the monocytes, but have nothing to do with the neutrophil series They regard the neutrophil myelocytes as stem cells for the neutrophil series We have been able to observe transitions from promyelocytes to myelocytes and believe that such assumptions based on pronounced pathological states (monocytic leukæmia) cannot be applied to normal conditions. The differentiation of monocytes from promyelocytes is facilitated by the examination of supravital preparations after the method of Sabin with neutral red and Janus green, as suggested by Trautmann (1940) Like Vortisch (1938) we have employed this method to distinguish between monocytes and lymphocytes The trialists Doan, Cunningham and Sabin (1925), Schittenhelm and Ehrhardt (1925) Forkner (1932), Schilling (1933), Aschoff (1938), Schultz (1940), Engback Heerup and Thomsen (1942) and others, assume the existence of an independent developmental series of monocytes Schulten (1930) and De Weerdt (1939) found only few monocytes in [illegible] rrow, which Schulten thinks is due [illegible] ure with peri[illegible] od This is really a point again[illegible] of the med[illegible] gin of the monocytes from the m[illegible] Segerdahl [illegible] es that sternal puncture has [illegible] le towar[illegible] m of the origin of monocytes. [illegible] 37), Ro[illegible] d other believe that marrow bio[illegible] ions fav[illegible] elop [illegible]

of monocytes from myeloblasts, because transitions betwe reticulum cells and monocytes could not be observed. De Weer did, however, observe such transitions. Doan, Cunningham a Sabin (1925) demonstrated in neutral red and Janus green prepa tions that monocytes are the only cells to take up neutral red granu in a rosette arrangement. The fact that reticulum cells do not so is a point against the reticular origin of the monocytes. Our o view is that monocytes are derived from medullary and ext medullary reticulum cells In infectious diseases in spite of co siderable monocytosis in the blood (20% and more, see Case 46, p. 2 we have not found an increase of these cells in the marrow, a believe that the extramedullary development of monocytes, perha from the reticulum of lymph glands, is the more common proce A predominantly medullary production of monocytes may occ in monocytic leukæmia, but the difficulty of identification of t cells of the monocytic series in pathological conditions (leukæm must be borne in mind, especially as the cells may be atypical.



FIG 15 Monoblast with indented nucleus and nucleoli Cytoplasm agranular and basophilic. (× 1,400)



FIG 16 Promonocyte with loose clear chromatin and basoph cytoplasm with definite granu tion (× 1,400)

The most primitive form, the monoblast, is a big cell, 12 μ–16 with a round or slightly indented nucleus, which has 1–2 nucle about 0 1 μ–0·2 μ in size Its cytoplasm is dark or medium bl and has no granules. Perinuclear halo formation and commenci vacuolation can often be observed (Fig 15) The next maturati stage, the promonocyte, first described by Seemann (1930) a Forkner (1932), is slightly larger, 16 μ–20 μ, and usually has indented nucleus with a fine chromatin network The cytopla is greyish blue and has azure granules (Fig 16)

The granules are usually markedly localized in the nucle hilum (Thaddea and Bakalos, 1939) and thus corresponds to t collection of neutral red granules seen in Sabin's supravital p parations Hittmair (1942) regards the promonocytes as one of t stem cells of the monocytes and distinguishes them clearly from t paraforms of the promyelocytes, but he traces the promonocyt back to the myeloblast Forkner (1932) demonstrated that t

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of monocytes from myeloblasts, because transitions between reticulum cells and monocytes could not be observed De Weerdt did, however, observe such transitions. Doan, Cunningham and Sabin (1925) demonstrated in neutral red and Janus green preparations that monocytes are the only cells to take up neutral red granules in a rosette arrangement. The fact that reticulum cells do not do so is a point against the reticular origin of the monocytes. Our own view is that monocytes are derived from medullary and extramedullary reticulum cells. In infectious diseases in spite of considerable monocytosis in the blood (20% and more, see Case 46, p. 284) we have not found an increase of these cells in the marrow, and believe that the extramedullary development of monocytes, perhaps from the reticulum of lymph glands, is the more common process. A predominantly medullary production of monocytes may occur in monocytic leukæmia, but the difficulty of identification of the cells of the monocytic series in pathological conditions (leukæmia) must be borne in mind, especially as the cells may be atypical.



FIG 15 Monoblast with indented nucleus and nucleoli Cytoplasm agranular and basophilic (× 1 400)



FIG 16 Promonocyte with loose nuclear chromatin and basophilic cytoplasm with definite granulation (× 1,400)

The most primitive form, the monoblast, is a big cell, 12 μ–16 μ, with a round or slightly indented nucleus, which has 1–2 nucleoli about 0 1 μ–0 2 μ in size Its cytoplasm is dark or medium blue and has no granules Perinuclear halo formation and commencing vacuolation can often be observed (Fig 15) The next maturation stage the promonocyte, first described by Seemann (1930) and Forkner (1932), is slightly larger, 16 μ–20 μ, and usually has an indented nucleus with a fine chromatin network The cytoplasm is greyish blue and has azure granules (Fig. 16)

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cytoplasm sometimes shows processes not unlike pseudopodia. The mature forms, or monocytes, 12 μ–20 μ, are well known in the peripheral blood by their loose pleomorphic nucleus, rather poor in chromatin, and by their light greyish-blue "frosted glass" sparsely granulated cytoplasm (Fig. 17)

Few lymphocytes and lymphoblasts are found in the fluid from marrow puncture. They are derived from the germ centres of the marrow and some are due to mixture with peripheral blood. Many authors regard the number of lymphocytes as an indication of the degree of dilution of the marrow with blood. Askanazy (1915) was the first to report the presence of lymph nodules in the marrow, but Hellman (1935) and Williams (1939) were unable to confirm their constant occurrence. Williams found them in only 30% of healthy subjects. It must be conceded that it is impossible to examine the entire amount of marrow in the body and thus occasional lymph nodules may escape observation.



FIG. 17 Mature monocytes (× 1,400)

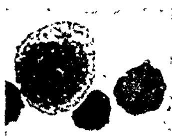


FIG 18 Lymphoblast with finely meshed nucleus and nucleoli Also some lymphocytes (× 1,400)

The differentiation of lymphoblasts and myeloblasts is not always an easy one. Rich, Wintrobe and Lewis (1939) distinguished them in tissue culture by the manner of their movements. Myeloblasts crawled in a long sausage-shaped sort of way and showed pseudopodia at their front end, but lymphoblasts moved in a shape not unlike a hand mirror, and also had pseudopodia at the front, but the rest was trailed behind like a tail. Monocytes continued to send out pseudopodia in all directions The authors recorded these movements by cinematography Thus tissue culture can contribute to the settlement of problems of development and of classification. De Bruyn (1944), however, could not confirm this work The value of mitotic figures in differential diagnosis will be discussed later The lymphoblast closely resembles the myeloblast, it has an equally fine chromatin structure, but a smaller number of nucleoli and its cytoplasm is less basophilic (Fig 18)

The chromatin structure is less differentiated than in the myelocyte It seems to be viscous, homogeneous and deep purple,

and the nucleoli are not at all distinct. In acute lymphatic leukæmia we have also observed cells with pale, finely reticular nuclei, which are apparently related to reticulum cells. Mature lymphocytes have a compact, coarsely trabecular nucleus and a light blue cytoplasm, which occasionally contains azurophil granules.

The Megakaryocyte Series

The marrow giant cells have also been divided into primitive and mature forms. The megakaryoblasts (Figs. 19 and 20), according to Barta (1932), are distinguished by a small rim of non-granular cytoplasm and an oval nucleus with net-like chromatin structure and an early tendency to pleomorphism. They are hardly larger than the myeloblasts. Frey (1928) and Rohr (1940) estimate that they constitute up to 5% of all marrow giant cells. Promegakaryocytes are larger, round or oblong, their nuclei are often kidney shaped, occasionally already pleomorphic and their cytoplasm is less basophilic than that of the megakaryoblasts and is partly granulated (Fig. 21). They form 4%–12% of the giant cells. The mature megakaryocytes are very large cells (40 µ–70µ) and show an irregularly lobed nucleus and a weakly basophilic cytoplasm with azurophilic granules (Fig 22 and others in Chapter X). Heilmeyer (1942) uses a slightly different terminology. His promegakaryocyte corresponds to our megakaryoblast and he estimates its frequency in 100 marrow giant cells at 0 8% (0%–3 3%) He also distinguishes non-platelet-forming megakaryocytes, which group also contains a certain number of the promegakaryocytes of our nomenclature (43%–79%), platelet-forming megakaryocytes (18%–38%) and loose nuclei (5%–15%) The platelet-forming marrow giant cells include all cells with even the slightest sign of platelet differentiation Heilmeyer estimates the total number of marrow giant cells as 0 4% (0 19%–0·78%) We believe that this terminology has definite disadvantages, in so far as morphology is identified with function Small clumps of platelets may be found very close to megakaryocytes and may actually simulate thrombocytogenesis

The marrow giant cells undergo a development which, as far as the staining reaction of the cytoplasm is concerned, corresponds to that of the myeloid series, i e., decrease of cytoplasmic basophilia, but as regards cell size they develop in the opposite direction, i.e., cell size increases with maturation, (Leitner, 1944). In addition to the forms already discussed, Seeliger (1924) describes degenerative forms with hyaline or filamentous and streaky cytoplasm and dark, pyknotic nuclei Their number amounts to 26% of the total megakaryocytes according to Frey (1928), and 28%–30% according to Rohr (1940), but we have usually found less than 20% and generally about 10% Galinowski (1938) in addition dis-



Fig. 19. Megakaryoblasts with homogeneous basophilic cytoplasm. (× 1,400)

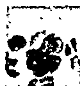


Fig. 20. Megakaryoblast with lobed nucleus (× 500)

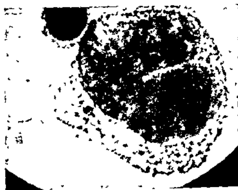


Fig. 21. Promegakaryocyte with finely meshed nucleus and basophilic cytoplasm, with commencing granules (× 1,400)



tinguishes a metamegakaryocyte, which is alleged to take part in platelet formation. We do not consider the introduction of such an intermediate type as desirable, because its morphological definition is uncertain, and it unnecessarily complicates the classification of the marrow giant cells. There is no evidence for di Guglielmo's (1925) view that the megakaryocytes with lobed nucleus are formed by the fusion of several cells with round nuclei (Lambin and Lammers, 1926; Wuyts, 1931; Japa, 1943; Leitner, 1944; and others). Japa (1943) has shown that all nuclei within the cell undergo mitotic division synchronously. The problem of the origin of platelets from the marrow giant cells will be discussed in the chapter on thrombocytopoiesis.

Marrow Reticulum Cells

The cells of the marrow reticulum have received attention only recently. Their number in marrow material is normally small, as they seem fixed in the tissues. Their identification is made difficult if they are separated from their surroundings. Before the introduction of marrow biopsy they were subdivided by morbid anatomists into "storage cells" (histiocytic macrophages of Maximow) and indifferent mesenchymal cells, which have no phagocytic function, but only the capacity to form various cells. Fleischhacker (1941) believes that the phagocytic reticulum cells proper are the only ones to belong to the marrow reticulum, though he mentions also other reticulum cells as occurring there Most writers accept Rohr's (1938) view, who distinguishes three varieties of marrow reticulum cells.

(1) Phagocytic reticulum cells, corresponding to Maximow's macrophages

(2) Large and small lymphoid reticulum cells.

(3) Plasma cells.

According to Fleischhacker (1941) the large lymphoid reticulum cell is derived from the undifferentiated reticulum cells, and quite often becomes a phagocytic or fat-storing reticulum cell He states that the small lymphoid reticulum cell is rather rare and also originates from the undifferentiated reticulum cell We believe that the small lymphoid reticulum cell is of little practical importance Apart from the fact that this cell cannot always be distinguished from the lymphocytes and its existence is, therefore, not proven, we do not know of any pathological condition which is accompanied by an increase in these reticulum cells The existence of the large lymphoid reticulum cell also is not universally accepted De Weerdt (1939) thinks it is a damaged fixed reticulum cell Heilmeyer (1942) believes that all primitive reticulum cells belong to this group as long as they are not granular We agree with Rohr that the marrow plasma cells belong to the reticulum group Plasma

cells are a separate species of cell, whose existence we were able to prove by photomicrography in our observations on marrow (see Plate, p. 48). Their developmental series can be traced back to a primitive form with specific nuclear characteristics, namely the plasmoblast (see later). This immature form in turn is derived from the reticulum cells or undifferentiated *histiocytes*, and we agree with Wegelin (1943) in this assumption. We subdivide the reticulum cells as follows .—

1. Primitive Reticulum Cells

These cells correspond most closely to Rohr's large lymphoid reticulum cell, but show no lymphoid characteristics. They probably have a hæmopoietic function in normal conditions. On the other hand in certain pathological states, especially in panmyelophthisic anæmias with an increase of reticulum, and in the reticuloses, the cells apparently lose their generative power. In the normal marrow primitive reticulum cells are rarely seen. They may be mistaken for artefacts or for damaged or squashed cells. In the disorders mentioned above they occur quite frequently and may even dominate the picture (*Fig.* 23).

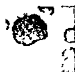
It is possible that their proliferation, for instance in anæmia, should be considered to be a compensatory measure, though an ineffectual one since they lose their ability to produce blood cells. Morphologically they are not difficult to recognize. They are cells with rather large round or elongated nuclei (7 μ-8 μ) with a spongy vesicular, chromatin structure, staining lightly with the usual Romanowsky-Giemsa method. The cytoplasm is light blue and as a rule non-granular, but at times it contains sparse fine granules. Often, however, especially when they occur in tissue-like cell collections, they are not clearly recognizable.



Fig. 23 Primitive marrow reticulum cells with juicy, spongy nucleus and nucleoli and basophilic cytoplasm ($\times$ 1,000)

The so-called *Ferrata cells* deserve mention at this stage. They are among the cells which have caused heated discussion. Ferrata (1935) believed that they are histiocytic elements (hæmohistioblasts) which may become blood cells, either directly or by way of the stage of the *hæmocytoblast*. Naegeli (1931) and Klima (1938) think they are really artefacts. Lambin (1927), Ringoen (1927), Segerdahl (1935), Schulten (1939), de Weerdt (1939), Thaddea and Bakalos (1939), and others regard them as squashed myelocytes, and Heilmeyer (1942) believes they are promyelocytes following abnormal maturation. In favour of the last opinion Segerdahl finds that these cells cannot be observed either in histological

sections or in the counting chamber We have pointed out that neutrophil and eosinophil myelocytes with squashed nuclei and damaged cytoplasm are seen occasionally and that they do show a spongy nucleus not unlike Ferrata cells On the other hand we have observed certain cells in myelopathies, which we regarded as primitive reticulum cells. They showed no evidence of squashing or other damage and had a nuclear structure, such as is described for Ferrata cells. Kienle (1943), who fully shares Ferrata's views, found them repeatedly in leukæmic marrow, and he concluded that their occurrence should arouse at least a suspicion of leukæmia. We cannot confirm this, but we firmly believe that a number of cells included among the Ferrata cells in many counts are in fact primitive marrow reticulum cells In our cases of myelopathies there was inhibition of maturation at a very early stage The nuclei



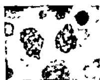




FIG 24 FIG. 25 FIG 26.

FIG. 24 Primitive marrow reticulum cell with loose nucleus and small rim of basophilic cytoplasm (× 500)

FIG. 25 Primitive marrow reticulum cells with double nucleus (dissociation of nucleus cytoplasm division) (× 500)

FIG 26 Primitive marrow reticulum cell with two nuclei and much cytoplasm (× 1,000)

were loose, juicy and lightly staining whilst the cytoplasm was pale blue, often with azurophil granules (Fig 24) Corresponding to severe marrow damage, atypical mitotic figures with duplication of the nucleus could be observed, and the nuclear structures clearly showed the features already described (Figs 25 and 26).

These cells can probably be classified as hæmohistioblasts, though in our cases a hæmopoietic function could not be proved Morphologically they belong to the primitive marrow reticulum cells or are very closely related to them. It is doubtful if they are normal stem cells, for our observations tend to favour the view that they are a stem cell that has undergone pathological change The primitive reticulum cells may possibly be hæmopoietic, so that the term hæmohistioblast [illegible]

represents an intermediate stage in the development of the reticulum

cell to the hæmocytoblast. But as we lack proof for this assumption, the term "primitive reticulum cell" is best used as it is based on morphological appearances.

2. Phagocytic Reticulum Cells

Unlike Fleischhacker (1941), we do not trace the phagocytic reticulum cell back to the primitive reticulum cell, but directly to the mesenchymal cell of the bone marrow, so that both cells are on the same developmental level, but not in a developmental line. Their existence is recognized by most authors, Mallarmé (1937),



FIG 27



FIG 28

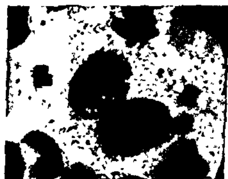


FIG 29

FIG 27 Phagocytic reticulum cell with slight phagocytosis of pigment (× 500)

FIG 28 Primitive phagocytic reticulum cell with spongy nucleus and four nucleoli The cytoplasm contains phagocytosed material (× 1,400)

FIG 29 Phagocytic reticulum cell with double nucleus and pigment phagocytosis. (× 1,400)

Markoff (1937), Skouge (1937), Henning (1938), Klima (1938), de Weerdt (1939) Rohr (1940), Fleischhacker (1941), Tischendorf (1941), Kienle (1943), Thaddea (1943), Leitner (1944), and others They have a relatively small nucleus, which is round and usually in an eccentric position, and a bluish hazy cytoplasm, which may contain granules, pigment or fat particles Less often the nucleus is larger or even lobed (Figs 27, 28, 29)

Révol (1938) follows Maximow (1932) by calling them macrophages Markoff (1937) attempted to estimate the proportion of

phagocytic reticulum cells by intrasternal injection of Indian Ink in moribund patients, but the figures obtained cannot be considered really normal data, as the composition of the marrow begins to alter with the onset of death. He also observed that lymphoid reticulum cells may become transformed into the phagocytic variety. Nordenson (1938) repeated Markoff's experiment with Indian Ink and noted that phagocytosis took place in 11% of reticulum cells. This rather small proportion of phagocytic reticulum cells contradicts Klima's (1938) view and the opinion of others, who consider all reticulum cells phagocytic. It must be remembered that phagocytosis may be facultative and is not always recognizable in all cells. The "empty" cells in a stage of quiescence are not always easily classified and this may explain Markoff's observations of the transition of lymphoid reticulum cells into phagocytic ones. It seems to us most likely that quiescent phagocytic cells have been interpreted as lymphoid ones by many workers.

3. Fat Storage Cells

Fat cells are reticulum cells which store fats or lipoids, and the



Fig. 30



Fig. 31



Fig. 32

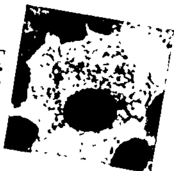


Fig. 33



Fig. 34

Fig. 30. Fat cell with much fat (× 1,400)

Figs. 31–34. Fat cells with varying degrees of storage. In Figs. 31, 32 and 33, cells, such as one might find in Gaucher's disease (foam cells) (× 1,400)

are in a group by themselves. They are rarely seen in normal marrow (0·1%), but *their number is increased* in disorders of the storage mechanism (Gaucher's disease, Niemann-Pick's disease, Xanthomatoses) We have often seen them in diabetes mellitus They are large cells (30 μ–80 μ) *with a relatively small nucleus*, which is often flattened and pushed to one side. When these cells are in a state of rest the nucleus may be central. The fat contained in the cytoplasm is dissolved by *alcoholic stains and disappears, so that the cytoplasm* presents a light, wide-meshed appearance ("foam cells" of Rohr) with feebly staining broad cords of condensed cytoplasm. The cytoplasm of the Gaucher cells, *according to Klima (1938), resembles* crushed tissue paper, and we have observed this appearance also in other fat cells in marrow (Figs. 30–34).

4. Endothelial Cells

Endothelial cells also occur in very small numbers in normal marrow They have round thick nuclei, with a loose chromatin network. Their cytoplasm is irregular, plentiful, and stains pale blue They are possibly derived from small blood vessels, which have been injured by the puncturing needle. Rohr (1940) does not classify them with the reticulum cells as they are already differentiated and have no recognizable relationship to reticulum Fleischhacker (1941) states that they are almost identical with reticulum cells, and cannot be distinguished from them with certainty, except when they appear elongated, following their separation from capillary endothelium. *Our observations* confirm this view. Their identification is made somewhat easier, when they are found in tissue-like groups of three or four cells.

5. Plasma Cells

The plasma cells and their nature are still matters for controversy. As we have already stated in a paper on glandular fever (Leitner, 1940), we distinguish medullary or reticular and lymphatic or glandular plasma cells. It is possible to draw this distinction in normal cases in material aspirated from lymph glands *and also from marrow* The difference becomes still more evident in pathological states, as for example in glandular fever, where numerous early and mature forms of plasma cells reach the blood stream The early forms, of which many generations may be observed in glandular fever, are larger than the marrow plasma cells and their nuclear chromatin structure is *a finer meshwork than in the marrow* plasma cells There are often transitional forms tending towards the large lymphocytes In infectious diseases, plasma cells are frequently *released from* lymph glands into the blood stream, and we have seen them in both glandular fever

and in infectious hepatitis. Moeschlin (1940) also demonstrated them in cases of rubella where there is often an increase of plasma cells in the blood without an increase in the marrow. He thus believes that, except in myelomatosis, the plasma cells do not come from the marrow, but we do not agree on this point. Markoff (1937) has described a case of serum sickness, in which he found an increase of plasma cells at first in the marrow and a day later in the peripheral blood. In such cases it is very difficult to decide whether one is dealing with medullary plasma cells or the lymphatic or glandular variety. The fact that the distinctive features seen in life are impossible to recognize in post-mortem material, is not an argument against subdivision of the plasma cells, since it is agreed that such morphological refinements are difficult to demonstrate in histological material But the distinction is important morphologically as well as theoretically when dealing with the release mechanism of the cells, and seems fully justified, even though we assume that the primitive forms of both varieties of plasma cells develop from the reticulum cell. Thus plasma cells may develop from the marrow reticulum cell as well as from the reticulum cell of the lymph glands In our present state of knowledge, it is impossible to say whether their morphological differences are due to their place of origin or mode of production, and we must assume a close relationship between both varieties of plasma cells. We recognize the following stages in the development of plasma cells :—

(a) **The Plasmoblast.** This cell is the most primitive form following the undifferentiated reticulum cell and is larger than the mature plasmocytes (20 μ–30 μ) Its nucleus is almost central and does not possess a coarse structure arranged in spokes, but has a fine chromatin network with one or more nucleoli It is usually larger than in the plasmocytes. The nucleus-cytoplasm ratio differs from that in the myeloblast, because a broad rim of cytoplasm is usually visible. The cytoplasm stains a deep blue, often with slightly reddish patches Granules are always absent, but a perinuclear halo formation is usually present although vacuolation is not a common feature (Fig 35)

(b) **The Proplasmocyte.** Proplasmocytes have a more mature nucleus, which is usually definitely eccentric The nuclear structure has not yet become trabecular, or spoke-like, but the chromatin network is coarser than in plasmoblasts and nucleoli are not so common and are less well defined Their cytoplasm is always deep blue and homogeneous apart from the usual perinuclear halo. Vacuoles are almost always present In vigorous plasma cell reactions both immature stages of the plasma cell series may show a rather lighter cytoplasm and the nucleus-cytoplasm ratio alters *in favour of the nucleus (Figs 36–38)*

(c) **The Semi-mature Plasmocyte.** These cells have a smaller

nucleus in proportion to the cytoplasm, nucleoli are never present and their chromatin network has become coarser, and the arrangement in spokes in cartwheel fashion can often be recognized (Fig. 39)



FIG. 35



FIG. 36.



FIG. 37



FIG 38



FIG. 39



FIG 40



FIG 41



FIG 42

FIG 35 Plasmoblast with well defined nucleoli (× 1,000)
FIGS 36–38 Proplasmocytes in various stages of maturity (× 1,000)
FIG 39 Semi mature plasmocytes. (× 1,000)
FIGS 40–42 Mature plasmocytes (× 1,000)

(*d*) **The Mature Plasmocyte.** Mature plasmocytes have a typically eccentric nucleus, whose chromatin structure is compact, dark, trabecular and cartwheel-like The dark blue cytoplasm is vacuolated, and the perinuclear halo is hardly ever absent Small cells with pyknotic nuclei are aged forms (Figs 40–42)

The development of the lymphatic plasma cells is almost identical to that just described, only the early forms are larger and the intermediate stages more varied We agree with Thaddea and Bakalos (1940) that the recognition of the plasma cells in normal

conditions does not present any difficulties, but in pathological states very atypical forms may be met. This also accounts for the comparatively recent recognition of the nature of the myeloma cells by Wallgren (1920). The names of "paraplasmoblast" and "paraplasmocyte" have been coined for their pathological forms in analogy with the terms in leukæmia. In the normal plasma cell series the origin of the plasma cell is clearly established With Lachnit and Walterskirchen (1939), Moeschlin (1940) and other authors we trace the plasma cells back to the plasmoblasts, but Fleischhacker and Klima (1936) consider that they are derived from undifferentiated cells allied to myeloblasts and Apitz (1940) believes that they may originate directly from the myeloblast. Klima has demonstrated primitive plasma cells which appear to be identical with our plasmoblasts and proplasmocytes. Gluzinski and Reichenstein (1906), Downey and Stasney (1936), Askanazy and Dubois-Ferrière (1942), and others believe that plasma cells are derived from the lymphocytes, but more recent investigations show this to be unlikely. It seems certain that plasma cells are produced not only in the marrow, as assumed by Keilhack and Linck (1941), but also in extramedullary sites

The Function of Plasma Cells. Perlzweig, Delrue and Geschickter (1928) have described the hyperproteinæmia which occurs in cases of multiple myeloma Magnus-Levy (1933) thought the increased viscosity of the blood could be correlated with the hyperproteinæmia while Citron (1921) linked it with the presence of anti-complementary bodies in the serum, found in complement fixation tests Hopkins and Savory (1911), Citron (1921), Freund and Magnus-Levy (1932) correlated its presence with the quicker clotting of blood, Bauer (1935) and Gros (1935) with the positive Takata-Ara test, Fleischhacker and Klima (1936) with the positive Weltmann test.

Wuhrmann and Leuthardt (1938) and other investigators made more precise analyses of the fractions of the various proteins, using the methods of Butler and Montgomery, and found the increase of protein was chiefly due to the increased globulins. Keilhack (1943) showed in a review of the 84 cases of myeloma reported in the literature that the change in the albumen-globulin ratio of the plasma depended on the increase of the euglobulin fraction, and only rarely on an increase of the pseudoglobulin fraction. Apart from these normal proteins, pathological protein substances develop in myeloma, but not all of these have as yet been isolated (Lang, 1928, Wintrobe and Buell, 1933, Jacobson, 1935, McFarlane, 1935, Bing, 1937, v Bonsdorff, Groth and Packalén, 1938, Leitner, 1944) Apitz (1940) introduced the term "para-proteins" to describe these abnormal proteins v Bonsdorff, Groth and Packalén found that, in addition to the Bence-Jones proteoses, peculiar protein bodies, which may be crystallized, are produced,

cells during mitosis. Such immature cells do not usually take *part* in the process of phagocytosis. Thus the production of globulin by myeloma cells is clearly established.

[illegible]

or c [illegible]

[illegible] tinction between medullary and lymphoglandular plasma cells is possible as far as function is concerned, as only the medullary variety takes part in protein production In his cases of rubella with a plasma cell increase, the level of the blood proteins was normal, and in our cases of glandular fever we found normal serum globulin readings. Wegelin (1943), on the other hand, reported a case of bronchiectasis with an increase of plasma cells in the marrow, lymph glands, lungs and spleen, in which hyperproteinæmia and globulinæmia were found during life. On the basis of this finding, he presumes that there are no morphological or functional differences among the plasma cells originating from the various organs, and that all plasma cells produce protein bodies. Apitz, Keilhack, and Heinlein believe the number of plasma cells is normally too small to provide the body with globulins, but according to Wegelin all the plasma cells present in the body (gastro-intestinal canal, lymph glands, etc.) are sufficient for this function. We think it is likely that reticular plasma cells occur in organs other than bone marrow Here too, Case 31 (p.229) provides a link in the chain of evidence, as we have observed numerous intermediate stages between the atypical large "vacuolations" and the normal plasma cell "vacuoles." We wish to call them plasma cell droplets or plasma cell bodies. These intermediate stages favour the existence of a protein-producing function of normal plasma cells. Their normal "vacuoles" are in reality the secretions of plasma cells.

Summary. (1) Numerous investigations have established the technique of sternal puncture, have revealed its possible sources of errors, and shown the morphological and numerical composition of the marrow obtained (2) The question of the origin of individual cell species (platelets, plasma cells, monocytes and others) has been largely cleared up, as the several developmental stages and the transitional forms have been observed at their places of origin (3) Morphological studies and serum protein estimations have proved the importance of plasma cells and reticulum cells in globulin production (4) The physiology of blood protein production is important from the point of view of protein metabolism, as well as possibly for the theory of antibody formation, and probably in the origin of tumours (Wuhrmann).

Table 4 classifies the cells, morphology and function of the reticulum cell series. With regard to the plasma cells, we distinguish medullary (reticular) and lymphoglandular forms.

In pathological cases, numerous morphological deviations occur, especially in the presence of reticular reactions in panmyelopathies,

TABLE 4

Classification of Reticulum Cells

Cell species	Morphology	Function
(1) Primitive reticulum cell (hemohistioblast)	Lightly staining, vesicular nucleus with fine chromatin structure Light blue cytoplasm.	Normally hæmopoiesis. In myelopathies often lose their regenerative power.
(2) Phagocytic reticulum cell	Nucleus rather smaller, darker. Mostly finely meshy chromatin structure, but less juicy.	Phagocytosis of hæmosiderin, etc.
(3) Fat cells (Storage cells)	Nucleus usually compact, eccentric, cytoplasm like crushed tissue paper. ("Foam cells.")	Fat and lipoid storage.
(4) Plasma cells	Immature forms (plasmoblasts) with primitive nucleus and nucleoli Mature plasma cells with cartwheel nucleus. Cytoplasm deep blue.	Formation of certain protein bodies

anæmias and in myelopathies, and in the reticuloses. These atypical forms are probably primitive reticulum cells which have undergone pathological changes For most purposes the different varieties of cells described here appear adequate The cell morphology and cell function have been worked out to a considerable extent by marrow biopsies

The developmental series of individual cell species in human marrow is shown in the table of the origin of cells (p. 48).

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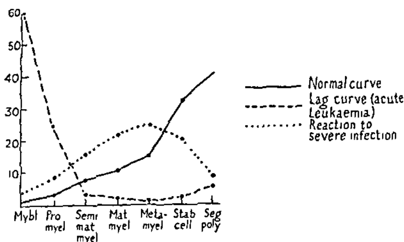
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CHAPTER V

MATURATION CURVES

MATURATION curves were first introduced by Pontoni in 1936 and Picena in 1937. They are graphic representations of the percentage figures of the individual maturation stages of the granulocytic or of the erythroblastic series. We agree with Fieschi (1938), de Weerdt (1939), Kienle (1942) and other workers that they are of definite value in reviewing a series of cases They show at a glance the morphological constitution of the marrow and allow conclusions to be drawn about the developmental tendency in each case. It is important to realize that where there is an increased demand for cells, the cells already present mature more rapidly, and only later is there increased proliferation, as shown by counts of mitotic figures In Graph 1, the percentages of the granulocytic series as found in twenty-two healthy subjects are charted as well as the main types of deviation from the normal, which we have observed in various cases



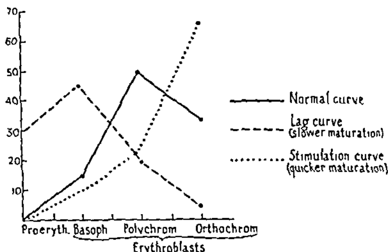
GRAPH 1 Maturation curves of the granulocytic series

Pontoni distinguishes the following deviations from the normal

(a) Curve of increased maturation rate characterized by increase of mature granulocytes, at the expense of the earlier forms

(b) Curve of slower maturation rate characterized by the increase of the more primitive forms at the expense of the mature forms This is commonest type met with Certain diseases e g, pernicious anæmia and leukæmia have characteristic maturation curves

Maturation curves have also been charted for the erythroblastic series. Graph 2 shows the percentage distribution of proerythroblasts, basophilic, polychromatic and orthochromatic erythroblasts in 22 normal cases.



GRAPH 2 Maturation curves of the erythroblastic series

Deviations from the normal may be distinguished as follows —

(*a*) Curve of speeded-up maturation with increase of the orthochromatic erythroblasts at the expense of the basophilic and polychromatic ones. This has been observed in post-hæmorrhagic anæmias, in pernicious anæmia undergoing liver therapy, and in regenerative anæmias Kienle states that such a curve is a favourable sign, but in our own experience this is only so when the total number of erythroblasts is not lowered

(*b*) Lag curves are characterized by an increase of basophil erythroblasts We have seen this in pernicious anæmia as an expression of the depression of maturation, and in hæmolytic anæmias as a consequence of increased regeneration As a prognostic sign such curves are only important in conjunction with a karyological curve, the peripheral blood picture and the reticulocyte response.

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CHAPTER VI

STUDIES OF MITOSES

MITOTIC figures can be observed in the peripheral blood only extremely rarely in certain severe blood diseases. *But with the* introduction of sternal puncture, mitoses can now be readily studied in the marrow at the site of origin of the cells. The examination of mitotic processes is of practical as well as of theoretical interest. Mitotic figures and their types differ in the various species of cells (*Ellermann, 1923; Petri, 1926; Hittmair, 1932;* Quattrin, 1941; Leitner, 1944), and help in differential cell diagnosis. The capacity of the blood cell system *to regenerate may be determined* from the number of mitoses, and more especially from the distribution of the various stages of karyokinesis. Karyological curves in conjunction with maturation curves provide valuable data for the interpretation of the myelogram in various disorders of *hæmopoiesis. Karyological curves do not run parallel to the* maturation curves, as proliferation (mitosis) and evolution (*maturation) are frequently dissociated Fieschi (1938) and Kienle* (1942), have made extensive studies of karyological curves The total number of mitoses is often increased The increased demand for mature cells is at first met by a speed up of maturation of the primitive forms in the marrow and only later by changes in the karyological curves and an increase *of the number of* mitoses. Picena (1937) expressed the number of mitotic figures per 100 cells of the same species and gave this quotient the name "Karyokinetic Index" Its value for white cells is 0 1%–1%, the average being 0 29%, and for erythroblasts 1%–4 6%, the average being 2·72% Kienle (1942) found 1 2%–2 1% (average 1 63%) for the red cells, Fieschi (1938) 1·2%–1·3%, and for white cells 0 5%–0 6%. We *agree with Fieschi and Kienle that mitoses in erythroblasts are* increased in hæmolytic jaundice, in polycythæmia, in pernicious anæmia treated with liver, and in many regenerative symptomatic anæmias, such as post-hæmorrhagic anæmia. The figure is decreased in toxic anæmias, such as from nephritis, malignant disease and certain infections, and in acute *myelopathies Kienle found it* increased also in anæmia from lead poisoning, which was the reverse *of our findings* De Weerdt (1939) found an increase in hæmorrhagic states

Fieschi (1938) elaborated a scheme for the plotting of karyological curves, based on observations by Politzer (1934) With Kienle, we distinguish six stages of mitosis —

(*a*) *Prophase with breaking up of the nucleus up to the compact* spireme phase

(*b*) Prophase with loose skein.
(*c*) Monaster phase (metaphase).
(*d*) Transitional stage to diaster phase.
(*e*) Diaster phase (telophase)
(*f*) Division of the cell body. (Dispireme phase.)

If it is not intended to plot karyological curves, the division into three main stages (pro-, meta- and telophase), is quite sufficient. The metaphase is best used for gauging the angle of mitosis (see below) The chromosomes are arranged regularly in the metaphase, whereas in the prophase they are in more or less compact skeins. In the telophase the chromosomes become coarser, less well defined and appear granular at times. Before discussing karyological curves, we would like to describe briefly the morphology of mitoses of the various maturation stages of cells.

Kienle has observed that hæmohistioblasts (Ferrata cells) in mitosis show smaller chromosomes than do the myelocytes. Myeloblasts in mitosis are found in marrow only rarely, according to Segerdahl and Fieschi and in our own experience Segerdahl (1935) puts their number at 0 029%, Fieschi (1938) at 0 02%–0 03%; but since normal marrow contains few myeloblasts only, the absolute number of mitoses in myeloblasts is high, being 2 18%. Fieschi (1938) and Quattrin (1941) report that the prophase shows a very voluminous spireme filling the entire cell In the metaphase and telophase, too, only a thin rim of cytoplasm can be seen. The star-form of the metaphase is regular, and individual chromosomes are distinguished only with difficulty. They are often swollen (Fieschi) probably owing to a tendency to degenerative changes. Quattrin found the chromosomes short and broad Telophases are rare, which would indicate a short and rapid development and probably also partial degeneration. Kienle (1942) assumes that many amitotic divisions occur, but we have not found any evidence of this He bases this assumption on the rarity of mitoses in myeloblasts

Mitotic figures are often seen in promyelocytes and myelocytes Segerdahl (1935) estimates the proportion of mitosing myelocytes at 0 069%, which corresponds to an absolute figure of 0·46% of the number of myelocytes as estimated by her Fieschi (1940) arrived at similar figures (0·1%, absolute figure 0 5%–0 6%), and Videbaek (1941) found a relative figure of 0 08%. This figure tallies with ours Whereas the prophase predominates among the myeloblasts, in the case of promyelocytes and myelocytes the metaphase and telophase are more common. The mitotic figures are characterized by a wide mitotic angle (68°–69°) The prophase does not entirely fill the cell, a definite broad cytoplasmic rim being present (Fig 44) In the metaphase and telophase the thick nuclear spindles are often arranged in beautiful monaster or diaster patterns (Figs 45–48) Kienle (1942) believes that in the

case of the promyelocyte, maturation proceeds during mitosis, thus explaining the preponderance of prophases. The granules in the cytoplasm make it easy to see whether one is dealing with promyelocytes, myelocytes (Fig. 49), eosinophils or basophils



FIG 43



FIG. 44



FIG. 45





FIG. 46



FIG. 47



FIG. 48

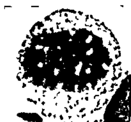


FIG. 49

FIG. 43 Mitosis (aster stage) of a marrow reticulum cell (× 500)

FIG. 44 Mitosis of a promyelocyte Prophase with compact skeins (× 1,400)

FIG. 45 Mitosis of promyelocyte Metaphase with wide angle of mitosis (× 1,000)

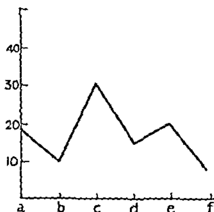
FIG. 46 Mitosis of promyelocyte, equatorial plate (× 1,400)

FIGS 47 and 48 Mitoses of promyelocytes. Telophases (× 1,400)

FIG. 49. Mitosis of a myelocyte Prophase with compact nuclear convolution, which does not occupy the cytoplasm entirely (× 1 400)

The mature forms from the metamyelocyte stage onwards do not undergo cell division

The number of chromosomes is forty-eight, but in pathological conditions numerous aberrations have been noted. Andres and Shiwago (1933) found changes in the structure of chromosomes in two cases of myeloid leukæmia. They noted swelling, partial fusion, apparent disintegration of chromosomes, multipolar karyokineses and abnormalities of the achromatic figure, and they considered these changes as evidence of the inferiority of such cells. Fieschi, however, does not think they are proof of the tumour-like nature of the leukæmias. We have found numerous atypical mitotic figures, especially dissociation of nuclear and cytoplasmic division, which will be discussed later (Leitner, 1944) Fieschi only rarely noted chromosomorrhexis, sometimes chromosomolysis in the prophase, but never aploidism or polyploidism, and only once multipolar karyokinesis. In judging qualitative changes in mitotic figures



GRAPH 3 Karyokinetic curve of the leucocyte series

we feel that caution is necessary, as such changes may be produced by the aspiration of the marrow or by the process of making the smear Undritz (1944) believes that the dividing process may continue until the smear has dried on the slide Graph 3 shows the distribution of the individual phases of mitoses in the granular cell series based on six normal cases It differs only very slightly from curves established by Fieschi and Kienle. In Graphs 3 and 4 (*a*) represents prophase with compact spireme, (*b*) with loose spireme, (*c*) and (*d*) the metaphase, and (*e*) and (*f*) the telophase

The erythroblasts have an acute angle of mitosis (18°–21°) and their chromosomes are short and stout The average frequency of mitoses is 1 83% for women and 1 17% for men (Fieschi) The number of chromosomes is forty-seven or forty-eight Divisions of proerythroblasts occur relatively rarely in normal bone marrow, but are more frequently found in anæmias during regeneration. The peak of proliferation by mitosis is among the early basophilic and polychromatic normoblasts In the case of proerythroblasts and also

in early normoblasts in the prophase almost the entire cell volume is filled by the spireme. The chromosomes are short and stout (Figs. 50 and 51). The megaloblasts keep their slender spindle form even in severe pathological states In metaphase their chromosomes are thinner than in normoblasts (Figs. 52 and 53).



Fig. 51







Fig 50 Fig 53 Fig 52

Fig 50 Mitosis of proerythroblast Prophase (× 1,400)
Fig. 51. Mitosis of proerythroblast, commencing equatorial plate. (× 500)
Fig. 52 Mitosis of promegaloblast Metaphase (× 1,400)
Fig 53 Mitosis of megaloblast Metaphase (× 500)

The chromosomes of the megaloblasts are plump only in the diasterphase Fieschi's asynchronism of nucleus and cytoplasm division is, in our opinion, not pathognomonic for megaloblasts. In promegaloblasts in the monasterphase the chromosomes are long and fine and easily counted, whereas in more mature forms they are crowded into the centre and much less well defined (Figs. 54–56).

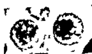






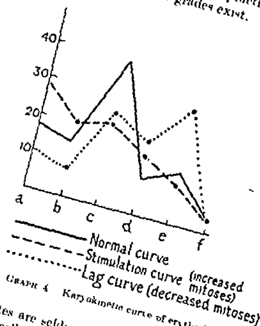
Fig. 54 Fig 55 Fig 56

Fig 54 Mitosis of normoblast Metaphase (× 500)
Fig 55 Mitosis of a normoblast Telophase Diaster (× 500)
Fig 56 Mitosis of a normoblast Completed division also of cytoplasm. (× 1,400)

Graph 4 shows the distribution of the individual phases of karyokinesis of erythroblasts. Apart from the deviations from the normal, Fieschi distinguishes:—

(1) Simple inhibition of karyokinesis, (2) total inhibition with decrease of all mitotic phases, (3) slowing down with decrease of the intermediate stages, and (4) stimulation with an increase of the speed of mitosis and of the prophases.

The distinction of two pathological types, the lag curve and the curve of stimulation, is sufficient for practical purposes, although, naturally, many intermediate grades exist.



GRAPH 4 Karyokinetic curve of erythroblasts.

Lymphocytes are seldom seen in mitosis and this is important in differential cell diagnosis. Their angle of mitosis is somewhere between that of myelocytes and erythroblasts, usually 38°–42°. We believe that only the primitive forms, i.e., lymphoblasts, and prolymphocytes undergo mitosis. Fig 57 shows mitosis of a lymphoblast from a case of lymphatic leukæmia.

Monocytes divide with short loops of chromosomes which show slight terminal clubbing.

The marrow giant cells undergo mitosis, but their division is only rarely observed. Megakaryoblasts have coarse, almost knotty chromosomes and a wide angle of mitosis, which is wider than in all other marrow cells. In the case of megakaryocytes it is often difficult to make precise observations owing to the size of the nuclear convolutions. Their chromosomes are thick and their angle of mitosis wide (Figs 58–60). All nuclei within a cell, if multinucleated, undergo division synchronously (Japa, 1943).

Mitotic figures in reticulum cells, excepting that seen by Kienle in a Ferrata cell already described (p. 37), have so far only been reported by us. We have observed them in cases of myelopathy with considerable reticulum cell hyperplasia (Fig 43)

Henning and Keilhack (1939) presume, and we agree, that plasma cells may undergo amitotic division. In sternal marrow of healthy



Fig 57.

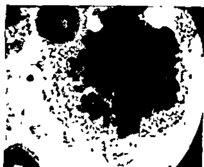


Fig 58.



Fig 59



Fig 60

Fig 57 Mitosis of a lymphoblast, metaphase (Case of lymphatic leukæmia) (× 1,050)

Fig 58 Mitosis of a megakaryoblast Prophase (× 1,050)

Fig 59 Mitosis of a megakaryoblast Metaphase (× 1,400)

Fig 60 Mitosis of a megakaryocyte Transition from prophase to metaphase (× 1,050)

subjects and especially in certain diseases, such as multiple myelomatosis, plasma cells with two, three or more nuclei have been seen In advanced cases of myelomatosis, where immature and atypical forms of plasma cells abound, multinucleated primitive plasma cells (plasmoblasts and proplasmocytes) have also been seen Their nuclei are usually rather big. We agree with Undritz (1944) that the presence of multinuclear cells is no proof of the amitotic division of plasma cells, but only a sign of the

dissociation of the division of nucleus and cytoplasm, such as described by Leitner (1941) in the case of myelocytes, in a case of myeloid leukæmia. In the peripheral blood and in puncture material from lymph glands in patients with glandular fever, mitotic figures in plasma cells have been found, and we believe, with Askanazy and Dubois-Ferrière (1942), Kienle (1942), Undritz (1944) and others, that the marrow plasma cells also divide by mitosis. In prophase they show a rather compact spireme with close loops, which occupies the greater part of the cell (Fig 61) In metaphase the star-form is often irregular whilst the chromosome loops are plump and relatively long (Fig 62) In telophase, clumping of the chromosomes is often but not invariably seen (Fig 63)

Normally rather fewer mitoses are found in the marrow than one would expect This fact is usually explained by the periodicity of the divisions Long quiescent periods alternate with short dividing phases Division of the white cells occurs only rarely at the stage of the myeloblast, but most often at the myelocytic stage (Leitner and Gugelot, 1938)

Andres and Shiwago (1933) use the following technique for their karyological studies: expose the preparation to osmic acid vapour, fix with Jolly's or Allen's mixture for ½–6 hours, wash with Chura's mixture, stain with toluidin blue, hæmatoxylin, azur-eosin or iron hæmatoxylin Whitby and Hynes (1938) prefer the dark ground method, which gives an appearance of greater maturity than is found in Romanowsky stained preparations In the interpretation of atypical pictures, caution is needed because Caffier (1928) has shown that considerable variations occur even in normal tissue Damage caused by the making of the smear must also be taken into consideration As regards the possibility of influencing mitoses experimentally, the reader is referred to a paper by Bujard (1944), who quotes all the relevant literature

Abnormal Cell Division

Abnormal mitotic figures are easily recognized They are usually signs of serious disorder and occur in large numbers only in grave blood diseases The following groups may be distinguished

Multipolar Karyokinesis Multipolar karyokinesis almost always points to a serious lesion In advanced pathological cases the mitoses may show three, four or even more poles Frequently the cells show evidence of degeneration as well

Degenerative Changes. Degenerative changes, such as disintegration of the chromosomes into granular fragments (chromosomorrhexis) clumping (pyknosis), fusion, and disruption of chromosomes should be considered to be of importance only if many damaged mitotic figures are seen They must be contrasted with multipolar divisions, which indicate a disorder of hæmopoiesis

present, even in small numbers only. The disruption of chromosomes, however, is usually pathological.

Fixation of Mitosis. Fieschi (1938) explains this phenomenon by a general tendency of the cells towards complete maturation and the consequent attempt to terminate proliferative processes as quickly as possible

Dissociation of the Division of Nucleus and Cytoplasm. This phenomenon is very frequently observed and is concerned in the problems associated with the question of amitotic division. The *suppression of cytoplasmic division following nuclear division* results in binucleated and multinucleated cells, which Schilling, Undritz and others have described as twins, triplets, quadruplets, etc We have observed them in panmyelopathy, in leukæmoid reactions, in severe infections and in myeloid leukæmia, and in nucleated red cells in severe anæmia. This tendency varies for different species of cells. Thus the phenomenon is found normally in plasma cells and occasionally in erythroblasts in normal marrow, but more frequently in moderate and severe anæmias. Granulocytes show the tendency almost exclusively in severe lesions of the marrow. There are even differences within the various varieties of granulocytes. The neutrophils show dissociation at an early stage, eosinophils show it later, and basophils later still. Megakaryocytes have little tendency to true multinucleation, although it has been described (Japa, 1943) The recognition of this phenomenon in megakaryocytes is often extremely difficult owing to the pleomorphism of their nuclei and the friability of their cytoplasm, which is easily damaged Caution is therefore necessary in the interpretation of these cells. Such dissociation, contrary to the view of Undritz (1944), should not be called malformation, which suggests an inherited cause This is a dissociation of the division of cells such as we have already described in the maturation processes in various disorders

Both maturational and divisional dissociation are of considerable diagnostic importance The two processes are probably closely related, as may be observed for instance in megaloblasts in pernicious anæmia Maturational and divisional dissociations do not, however, run parallel, nor are they as a rule consecutive A knowledge of the series of events affecting the tendency to multinuclearity of the various cell species is essential in order to interpret the dissociation of nuclear and cytoplasmic divisions Figs. 64–87 give examples of the great variety of forms found in sternal puncture material.

Like Undritz we have been unable to find any supporting evidence for amitotic division, although its occurrence is considered certain by Quattrin (1941), Kienle (1942) and others, and probable by Fieschi (1938). We have collected many photographs of cells similar to those seen by Kienle, but we do not think they furnish complete



FIG. 61.

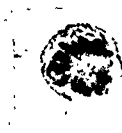


FIG. 62



FIG. 63



FIG 64.



FIG. 65



FIG 66



FIG 67

FIG 61 Mitosis of a plasma cell Prophase (Case of plasma cell leukæmia with Auer rods) (× 1,400)

FIG 62 *Mitosis of plasma cell Metaphase* (× 1,400)

FIG 63 Mitosis of plasma cell Telophase Diaster phase (Case of myelomatosis) (× 1,400)

FIG 64 Binucleated plasma cell in normal marrow (× 1,000)

FIG 65 Trinucleated plasma cell (Case of infectious hepatitis) (× 1,400)

FIG 66 Binucleated plasmoblast (Case of myelomatosis) (× 1,000)

FIG 67 Trinucleated plasma cell in plasma cell leukæmia (× 1,400)



Fig 68.

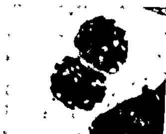


Fig. 69.

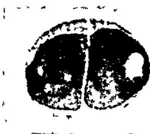


Fig. 70



Fig 71.

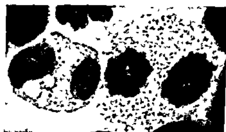


Fig. 72

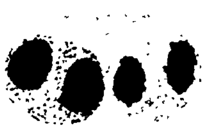


Fig. 73

Fig. 68 Quadrinucleated plasma cell in leukæmoid reaction (× 1,050)

Fig. 69 *Binucleated early normoblast in almost normal marrow* (× 1,400)

Fig 70 *Binucleated myeloblast in leukæmoid reaction* (× 1,400)

Fig 71 Binucleated promyelocyte (× 1,400)

Fig 72 Binucleated myelocyte and myelocytic mitosis in telophase, a case in favour of the mitotic origin of binucleation (× 1,400)

Fig 73. Binucleated myelocyte and myelocytic mitosis in telophase, cf Fig 72 (× 1,400)

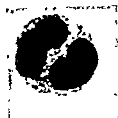


Fig. 74



Fig. 75.



Fig. 76.



Fig. 77.



Fig. 78

Fig. 74 Binucleated metamyelocyte in leukæmoid reaction. (× 1,400)

Fig. 75 Stab cell with two nuclei in leukæmoid reaction (× 1,400)

Figs 76 and 77 Trinucleated myelocyte with nuclei varying in size. (× 1,400)

Fig 78 Quadrinucleated promyelocyte in leukæmoid reaction; strongly vacuolated cytoplasm (× 1,400)



Fig. 79.



Fig. 80



Fig. 81



Fig. 82.



Fig. 83

Fig. 79 Promyelocyte with eight nuclei in leukæmoid reaction (× 1,400)

Figs 80 and 81 Quadrinucleated myelocyte with nuclei differing in size (× 1,400)

Figs. 82 and 83 Quadripolar mitoses, which give rise to quadrinucleated myelocytes (× 1,400)

proof for the existence of amitotic division. On the other hand, we have often observed stages preliminary to mitosis in dissociations of nuclear and cytoplasmic division, which left no doubt about the mitotic origin of multinuclear cells. Kienle himself describes such cells in pseudo-amitosis, where the mitotic agitation of the nuclei

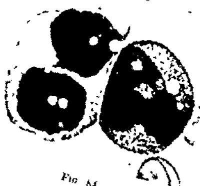


Fig 84

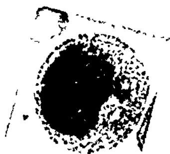


Fig 85

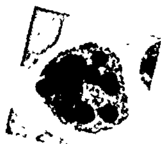


Fig 86



Fig 87

Fig 84 Binucleated myelocyte with fine thread joining the two nuclei (× 1,400)

Fig 85 Myelocyte with a small additional nucleus in leukæmoid reaction. (× 1,400)

Fig 86 Multinucleated eosinophil in leukæmoid reaction (× 1,400)

Fig 87 Binucleated megakaryocyte (× 1,400)

(prophases of mitosis) is easily recognized The connection of double nuclei with fine nuclear threads is not unusual in mitotic divisions Now and again we have seen even broader bands between the nuclei of multinucleate cells However, the fact that such cells have also been observed in a state of mitosis does not provide evidence in favour of their amitotic origin Hence judgment should still be reserved on this phenomenon

In any one case, all kinds of abnormal mitoses described above may be found. In certain blood disorders involving damage *to cell* production, dissociation of the nuclear and cytoplasmic division occurs early as seen in the marrow, so that multinuclear *cells are produced owing to a suppression* of cytoplasmic division. Moderate damage apparently results in binuclear cells, and severe damage causes the production of trinuclear and multinuclear cells, following tripolar and multipolar mitoses. In other cases, for instance in the leukæmias, we find degenerative changes of the chromosomes and of the nuclei in the early phase of mitosis, whereas divisional dissociation may be seen only rarely or not at all.

The following rules may be formulated :—Where multinuclear cells predominate with little or no change in the chromosomes, the dissociation of the nuclear and cytoplasmic maturation is the primary *process This may occur, as already described, in plasma cells without recognizable damage. Where changes in the chromo*somes dominate the picture in the presence of few or no multinuclear cells, the change in the chromosomes may be considered to be the primary cause of damage. These observations tend to favour the assumption that there are probably noxious factors, which damage mainly the cytoplasm or alternatively the chromosomes, thus apparently producing divisional dissociation or abnormalities in the chromosomes

Summary. Knowledge of the mitotic forms and the progress of mitosis allows certain conclusions about cell proliferation to be drawn The examination of marrow obtained by biopsy not only reveals a momentary glimpse into marrow composition, but also gives a view of the future development of the marrow picture. Plotting of karyological curves appears to serve a useful purpose in certain cases Observations on sternal marrow with multinuclear *cells favours the theory of mitotic division of blood and marrow* cells No conclusive evidence proving amitotic division is available so far. Nuclear and cytoplasmic division is never synchronous as cytoplasmic division follows division of the nucleus In dissociation of nuclear and cytoplasmic division the stage of division of the cytoplasm is not reached Such deviations from the normal type of cell division always indicate severe pathological states in the granular cell series In the erythroblastic series they indicate less severe damage, and in the case of plasma cells they suggest profound disorder only when seen in appreciable numbers Trinuclear, quadrinuclear and multinuclear cells should be considered rather more serious pathological signs than binuclear cells, because they originate from tripolar and multipolar mitotic figures Multipolar karyokinesis can be found as a rule in severe disorders of the blood, especially in the granular cell series Deviations from the normal type of chromosomes are observed either alone or together with dissociation of the nuclear and cytoplasmic division.

CHAPTER VII

THERAPEUTIC TRIALS BY STERNAL PUNCTURE

JOSEFSON (1934) and Roversi and Tanturri (1935) were the first to suggest intrasternal injection of Campolon in pernicious anæmia. Since, however, this preparation is equally successful when given by intramuscular injection, this method was soon abandoned. Other preparations designed to produce a storage effect were used by Codvelle, Bernard and Guichené (1936), Picena (1937) and Tocantins (1940), by using Congo red and fluorescin, found that these preparations, injected intrasternally into the marrow, were rapidly absorbed into the blood stream. Henning (1940, 1943) recommended blood transfusion by sternal puncture, especially in patients with poor veins. This method has been found valuable by many authors (Giraud and Desmonts, 1941; Battistoni, 1942; Heinrich, 1942; *Sousa Dias, 1942; Erhardt and Kneip, 1943; Junghanns, 1943;* König and Drasnar, 1943; Roith, 1943; Bailey, 1944, Fuchs and Denber, 1944, Gimson, 1944) We have only made use of intrasternal transfusion in one case, where it was tolerated well Tocantins, O'Neill and Jones (1941) shortly after Henning's first publication suggested intramedullary transfusions. In children they use the tibia, in adults the sternum. Henning prefers the sternal route even in infants, where he introduces the needle somewhat obliquely. Other workers have made use of marrow obtained by sternal puncture for therapeutic purposes Schretzenmayr (1937) was the first to inject marrow from sternal puncture intramuscularly in anæmias. Like other workers we have not been convinced of the value of this method, even when marrow was given by intramedullary injection (Moeschlin and Rohr, 1944) Morrison and Samwick (1940) have *employed intrasternal bone marrow injection successfully in two cases of aplastic anæmia,* but 2 cases treated similarly by Britton (1945) were completely unsuccessful From the practical point of view it may be stated that intrasternal transfusions, infusions, and injection of medicinal preparations have probably got a definite place in those cases, where *for one reason or another intravenous* administration appears contraindicated or impossible Many workers have found blood transfusions by this method technically difficult, whereas infusion of plasma or salines is simple and almost ideal in many cases

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CHAPTER VIII

DISORDERS OF ERYTHROPOIESIS

MEGALOCYTIC ANÆMIAS

Pernicious Anæmia (Addison's Anæmia, Biermer's Anæmia)

PERNICIOUS anæmia is one of the diseases most frequently investigated by sternal puncture. The earliest marrow biopsies were made by Zadek (1921) and Peabody (1927), who trephined the tibia Fontana (1928) used Seyfarth's sternal trephine method. Schilling was the first to recognize that the "lymphoid marrow," so called by Naegeli, was in fact an erythroblastic marrow.

It is generally agreed that untreated pernicious anæmia shows a typical marrow picture characterized by great cellularity and by promegaloblasts and megaloblasts (Barta, 1931; Schilling, 1933; Segerdahl, 1934, Henning, 1935; Nordenson, 1935; Rohr, 1935; Roversi and Tanturri, 1935; Mallarmé, 1936; Markoff, 1936; Picena, 1937, Schulten, 1937, Storti, 1937, Fieschi, 1938, Heilmeyer, 1938, Klima, 1938, Komiya, 1938, Penati and Saita, 1938, Révol, 1938, Weil and Perlès, 1938, Scott, 1939; *Thaddea and Bakalos, 1939; De Weerdt, 1939; Leitner, 1941*, Kienle, 1943). The severity of the anæmia is reflected in the marrow picture. It is proportional to the increase of primitive cells (promegaloblasts), and the increase of megaloblasts at the expense of normoblasts (Cotti, Balestieri and Volta, 1938; Penati and Saita, 1938; as well as previously quoted authors). Penati and Saita state that megaloblasts first appear when the number of erythrocytes has fallen to 3 5 millions per cmm This fact makes *sternal punctures particularly valuable in the early diagnosis of* pernicious anæmia. De Weerdt and others consider that normoblasts continue to dominate the picture until the erythrocytes have fallen to 2 millions. *But as megaloblasts are specific for* pernicious anæmia, even small numbers are sufficient for diagnosis In our own cases the number of megaloblasts varied between 6% and 48% of [illegible] disease Za[illegible] Kaznelson ([illegible] of 15·75% in 24 cases, Doan and Zerfas (1927) 0 6%–14 4%, Nordenson (1935) up to 50%, Rohr (1940) up to twice the number of white cells, *Storti (1937) and Fieschi* (1938) 25%–50%, Orsi, Ramos and Tranchesi (1938) half of the number of erythroblasts Young and Osgood (1935) 16 8%, Piney (1933) 25%–40%, Custer (1933) 38·6%, Cotti, Balestieri and Volta *(1938) 12–51 per 100 white cells* Naturally these figures depend on the proportion of early cases in

the series concerned. The presence of megaloblasts and the marked erythroblastic hyperplasia as a whole make diagnosis easy in most cases. Oria, Ramos and Tranchesi found 100 erythroblasts, Rohr and Moeschlin (1942) found 200 erythroblasts, and we, ourselves, found 70–180 nucleated red cells per 100 white cells. Occasionally atypical cases are seen, with marrows relatively poor in cells, and we have seen two such cases. One case was also suffering from syphilis with a positive Wassermann reaction, and the other had tuberculosis. Only a certain number of promegaloblasts mature to the stage of megaloblasts, and of these again only a small proportion become erythrocytes without nuclei, and therefore the blood will show a deficiency in erythrocytes in spite of the apparently



Fig. 88

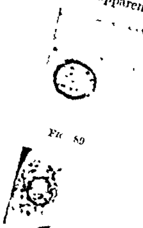


Fig. 89

Fig. 90

Fig. 88. Basophilic megaloblast and typical anisocytosis and megalocytosis in pernicious anæmia (× 1,000)

Figs. 89 and 90. Cabot's rings and punctate basophilia of erythrocytes in pernicious anæmia (× 1,400)

regenerative reaction in the marrow. While many authors report an increase in mitotic figures (in our cases 3.2%–6.7%), Fieschi (1938) in one case observed the almost normal figure of 1.6%, and at the same time noted a slowing down of the rate of mitosis.

Schulten (1937) considers that the formation from megaloblasts of the oval hæmoglobinized megalocytes, which are found in the peripheral blood has not yet been proved (Figs. 88–90). We consider that the early hæmoglobinization of the megaloblasts is a strong point in favour of the derivation of megalocytes from megaloblasts.

Schultz and Buding (1940) observed in sternal marrow obtained just before death from a severe case of pernicious anæmia that megalocytes and poikilocytes separated from megaloblasts and early normoblasts by a process of budding and Schultz (1944) has recently

reported similar findings. Habelmann (1940) believes that poikilocytes are derived from primitive erythroblasts. This is quite plausible, because poikilocytes occur in severe anæmias, and are *not at all specific* for pernicious anæmia. Brugsch (1934) and Wintrobe and Mitchell (1940) and others found an increase in the mean cell diameter of red cells during reticulocyte crisis. *Cotti* and Ciboldi (1940) believe this indicates maturation of megaloblasts into megalocytes, but this is not proved as it also occurs in the reticulocytosis following on acute hæmorrhage (Whitby and Britton, 1946) Schulten says that the prolonged presence of *megalocytes in the peripheral blood and at the same time* the early disappearance of megaloblasts from the marrow following liver treatment contradicts any connection between these two cell forms. This argument is not sound, because mature anuclear forms live longer than megaloblasts which are still capable of development under the action of liver treatment. Michelazzi (1943) has actually observed development of megaloblasts into normoblasts in blood (tissue) culture. Leitner and Gugelot (1938), Israëls (1939), Whitby and Britton (1946) and others believe in the existence of a separate promegaloblast-megaloblast-megalocyte series in untreated pernicious anæmia

With liver therapy megaloblastic marrow becomes transformed *rapidly into normoblastic marrow* This process commences within 24–48 hours, and is completed in a few days (Fontana, 1928; Barta, 1931; Tempka and Braun, 1932; Jones, 1934, Segerdahl, 1934, Nordenson, 1935, Henning and Keilhack, 1936, 1939; Markoff, 1936, Rohr and Koller, 1937; Schartum-Hansen, 1937; Schulten, 1937; Storti, 1937; Heilmeyer, 1938; Vischer, 1938; Koller, 1939, Leitner, 1941; Davidson *et al*, 1942; Kienle, 1943; Schleicher, 1945, Harrison and White, 1946; Levy, 1947; and others) Transformation of marrow precedes the reticulocyte crisis in the blood, which does not occur till the fourth day This, of course, is only natural, because reticulocytes are an intermediate stage between late normoblasts and erythrocytes While Storti (1937), Rohr (1940) and others deny the development of megaloblasts into normoblasts by liver therapy, the presence of enormous numbers of normoblasts 1–2 days after the commencement of liver treatment can only be explained by the transformation of promegaloblasts into cells belonging to the normal erythropoietic series *Davidson et al* (1942) showed that megaloblasts begin to disappear within six hours of effective treatment. This rapidity of change points strongly to the conversion of megaloblasts into normoblasts. Unlike De Weerdt (1939), Rohr was unable to observe intermediate stages between megaloblasts and normoblasts. But this is no proof that megaloblasts do not become transformed into normoblasts because liver therapy may turn promegaloblasts into proerythroblasts, and future maturation may then proceed from this

stage. On the other hand one would have to assume that under the action of liver extracts megaloblasts perish in a few days or a few hours (Davidson *et al.*) and that the normoblastic hyperplasia would have to take origin from the relatively few proerythroblasts.

Clinical and hæmatological remissions in pernicious anæmia and in the megalocytic anæmia of sprue have been effected with folic acid, the Lactobacillus-casei factor (Snell and Peterson, 1940; Stokstad, 1941), which is a pteroylglutamic acid. Remissions are similar to those produced by liver preparations. Doses of 15–20 mg orally or 20 mg. intramuscularly produce a reticulocyte crisis and a normoblastic change in the bone marrow (Berry and Spies, 1946; Wilkinson, Israëls and Fletcher, 1946). The results were exceptionally good when 5–10 mg. folic acid orally were combined with ½ unit of liver extract intramuscularly. Neurological damage can only be prevented by liver therapy (Meyer, 1947). Thymine (2, 4 dioxy-5-methylpyrimidin) in daily doses of 2·0–3·4 g. appears to produce similar results (Spies *et al.*, 1946), but the experience with this preparation is not so wide as in the case of folic acid.

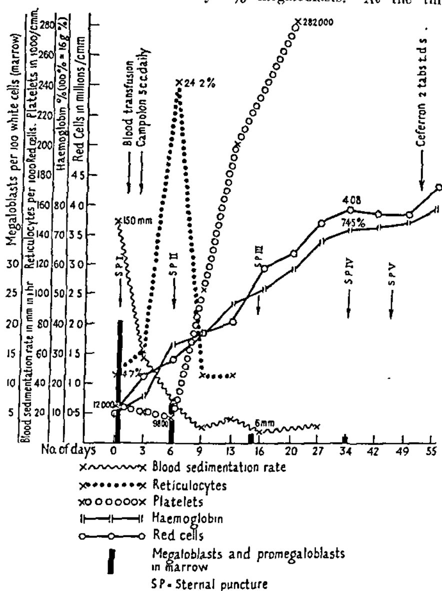
The following case illustrates how impressive transformation may be following liver therapy:—

Case 1. A woman of 53 years had measles and pertussis during childhood, rheumatic fever when 18 years, and gonorrhœa and syphilis when 51. Patient's mother died of anæmia at the age of 76. In the summer of 1936 she had an epistaxis, which recurred at approximately weekly intervals. At the same time she developed shortness of breath, pallor and œdema of the legs. Early in 1938 lassitude and dyspnœa increased. A stay in hospital failed to improve her. In September, 1938, there was further deterioration: cough with sputum, dyspnœa, lassitude, yellow skin, œdema and then a severe epistaxis (alleged loss about 1 litre).

On examination on admission: typical lemon yellow skin. No glossitis. Apical systolic murmur. Apex beat one and a half fingers outside nipple line. Liver edge level with the costal margin. Spleen palpable one finger's breadth below costal margin. Râles at both pulmonary bases. X-ray: considerable cardiac enlargement to the left. Pulmonary congestion. **Blood:** R.B.C. 612,000, Hb 21·6% = 3·5 g.%, C.I. 1·8, W.B.C. 5,920, 59% of which were segmented polymorphs, some with hypersegmentation. Platelets 9,800. Anisocytosis, poikilocytosis, polychromasia, 8% normoblasts, 0·5% early basophilic normoblasts, occasional Howell-Jolly bodies. Bilirubin in the serum: direct delayed reaction, indirect 0·75 mg. Alkali reserve 50 vol.%. Serum protein 5·34%, albumen-globulin ratio 65 : 35. Blood urea 29 mg.%, non-protein nitrogen 86 mg.%, cholesterol 86 mg.%, calcium 10·7 mg.%. Takata-Ara negative. Graph 5 shows the progress of the disease as well as the findings in five sternal punctures.

This was a severe case of pernicious anæmia. Marrow transformation was somewhat slow and the marrow picture was a little atypical, as cells were not very numerous, and in spite of the severity of the anæmia there were fewer promegaloblasts than megaloblasts. Similar observations have been made by Cotti, Balestieri and Volta

(1938). The myelogram was dominated by promegaloblasts (Figs. 91–93) and megaloblasts (Figs. 94–96). The second sternal puncture showed transformation into a normoblastic type of marrow (Fig. 97) with only 5% megaloblasts. At the third



GRAPH 5 Severe case of pernicious anæmia with return to normal following blood transfusion and liver therapy

puncture only occasional megaloblasts were found in the very cellular normoblastic marrow In the peripheral blood the reticulocyte crisis did not occur until the sixth day with 24·2% reticulocytes By this time the marrow had almost returned to normal In 4 weeks the number of erythrocytes rose from 612 000 to 4 08 millions and the hæmoglobin from 21·6% = 3 5 g % to 74·4% –



Fig. 91



Fig. 92.



Fig. 93



Fig. 94



Fig. 95



Fig. 96.



Fig. 97

Fig. 91 Promegaloblast megaloblastic *marrow in pernicious anæmia* (× 500)

Fig. 92 Promegaloblast and megaloblast in *sternal marrow in* pernicious anæmia (× 500)

Fig. 93 Promegaloblast and *megaloblast in mitosis (diaster phase)* in *sternal marrow in* pernicious anæmia (× 1,400)

Fig. 94 Megaloblasts in sternal marrow *in pernicious* anæmia. (× 500)

Fig. 95 Orthochromatic *megaloblasts in sternal* marrow in pernicious anæmia (× 1,000)

Fig. 96 Polychromatic megaloblast in sternal *marrow* in pernicious anæmia (× 500)

Fig. 97 Transformation of megaloblastic *into normoblastic* marrow *in* pernicious anæmia following liver treatment (× 500)

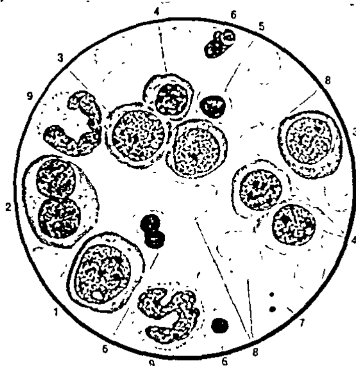
12 g.% At this stage sternal puncture revealed moderate hyperplasia of the normal erythropoietic series with 41·25 normoblasts, 2·5 early basophilic normoblasts and 1·5 proerythroblasts per 100 white cells In spite of this, no further progress was made; the number of erythrocytes even fell to 3 9 millions. Colour index was 0 92, the serum contained no bilirubin. The sedimentation rate fell from 150 mm. at 1 hour to normal. The high sedimentation rate may be explained by the extreme anæmia. Blood urea and non-protein nitrogen figures became normal. The albumen-globulin ratio was 80:20. As intensive liver therapy failed to bring complete recovery, iron was given in the form of Ceferron (two tablets three times daily), and the number of erythrocytes rose in 5 days to 4·5 millions and hæmoglobin to 82% = 13 2 g %

Towards the end of the primary treatment of pernicious anæmia the administration of iron often becomes necessary, as the rapid regeneration of hæmopoiesis exhausts the iron depots of the body, which may already be low owing to intestinal upsets caused by achlorhydria. Production of hæmoglobin often cannot keep up with the formation of erythrocytes. This may be shown by a low colour index and can always be detected by the presence of a low mean corpuscular hæmoglobin concentration. De Raadt (1942), Vahlquist (1942) and Brochner-Mortensen (1943) observed a fall in the serum iron content during liver therapy for pernicious anæmia. Waldenström (1944) states that this is a reliable and specific sign of pernicious anæmia Vannotti and Delachaux (1942) noticed a fall in the iron which is split off only with difficulty Adjuvant iron therapy is recommended by numerous authors (Beebe and Lewis, 1931; Sturgis, 1932, Franke, 1934; Murphy, 1934; Mogensen, 1935, Britton, 1936; Virkkunen, 1936; Schulten, 1937, Heilmeyer, 1938, Vannotti, 1940; and others). Markoff (1938) makes the interesting observation that when pernicious anæmia and hæmosiderosis co-exist, the reticulo-endothelial system becomes "choked" (Vannotti), and the iron level in the marrow consequently falls This abnormality of erythropoiesis cannot be corrected by liver The anæmia has become refractory to liver owing to the iron-deficiency and therefore iron must be given.

Case 1 is also instructive for other hæmatological changes. The considerable thrombocytopenia of 9,800 platelets and the slight leucopenia in the peripheral blood shows that the disease process is not confined to erythropoiesis in pernicious anæmia. Leucopenia with relative lymphocytosis and hypersegmentation of the neutrophils is a characteristic sign and indicates damage to the process of granulocytopoiesis, while lymphocyte formation in extramedullary regions remains unimpaired Hypersegmented neutrophils and large cells, which many authors have termed "neutrophil giant cells," are also seen in sternal marrow. The "giant stab forms" are said by Tempka and Braun (1932) to

PLATE I

Sternal Marrow in Pernicious Anæmia

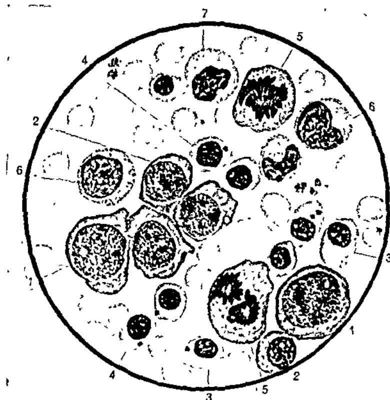


8 Typical oval megalocytes
9 "Giant neutrophil"

[To face page 78

PLATE II

Sternal Marrow in Hæmolytic Icterus, also illustrating the Normal Erythropoietic Series



1 Proerythroblasts with deep blue cytoplasm and a nucleus with fine meshwork and a nucleolus
2 Early basophilic normoblasts
3. Polychromatic normoblasts, some with Howell Jolly bodies.
4 Orthochromatic normoblasts, some with Howell-Jolly bodies.
5 Early basophilic erythroblasts in mitosis.
6. Neutrophil myelocytes
7. Neutrophil metamyelocyte

Scattered throughout the field are polychromatic and orthochromatic erythrocytes and some microspherocytes

originate from promyelocytes by missing intermediate stages Thaddea and Bakalos (1940) believe they are derived from more mature neutrophil cells These big cells (Figs. 98–100) have been reported in pernicious anæmia in sternal marrow first by Tempka and Braun, and later by Henning (1935), Thaddea and Bakalos (1940), Mallarmé (1937), Schulten (1937), Rohr (1938), Thaddea and Bakalos (1940), Leitner (1941), Kienle (1943) and others. Presumably this deviation from the normal relationship between nucleus and cytoplasm is caused by some disturbance of development Wilson (1942) has reported giant *stab* cells as the only abnormality in the marrow of patients with subacute combined degeneration of the cord not associated with anæmia

Many authors (Yamamoto, 1925; Doan and Zerfas, 1927, Dameshek and Valentine, 1937; Fieschi, 1938, and others) have observed disturbances of white cell maturation with an increase of myelocytes in the marrow Barta (1931), Segerdahl (1934), Henning (1935), Nordenson (1935), and Kienle (1943) report changes also in the myeloblasts, but like Fieschi we have not observed an increase in the size of myeloblasts



Fig. 98



Fig. 99



Fig. 100

Fig 98 "Giant neutrophil next to a normal segmented polymorph in sternal marrow in pernicious anæmia (× 500)
Fig 99 "Giant stab form" in pernicious anæmia (× 500)
Fig 100 Neutrophil twin cell (double stab form) in pernicious anæmia (× 1,000)

Fieschi has reported that promyelocytes also are usually normal in size or may be even small but we have seen large types of these cells Fieschi believes that large myelocytes and metamyelocytes indicate a speed up of maturation We have, like Nordenson, on several occasions observed toxic granulation indicating inhibition of the maturation of the cytoplasm, but Rohr (1940) disagrees on this point

Baserga and Gallo (1941) found evidence of disturbances of maturation in the mitotic figures of the white cells Karyokineses of myelocytes and of metamyelocytes were diminished Cell divisions were confined to myeloblasts and promyelocytes There are thus three signs of disturbance of the maturation mechanism of the granular cells (1) A myelocytic shift to the left, (2) a change in the mitotic picture, (3) the large neutrophils The twin cells (Fig 100) (Schilling) should be distinguished from the giant neutrophils (Fig 98).

After mitosis the division of the cytoplasm is suppressed, so that these cells have two nuclei and double the quantity of cytoplasm. This dissociation of nuclear and cytoplasmic division has already been discussed at length earlier in the book (pp. 62–68) Twin cells are quite common in pernicious anæmia. The difference in size of the giant neutrophils is not so striking: diameter of myelocytes 16·3 μ, metamyelocytes 14·9 μ, polymorphs 14·2 μ instead of the normal 14·3 μ, 12·7 μ and 12·9 μ respectively (Markoff, 1936, 1938). Hypersegmentation may represent a compensatory process, in which the body attempts to balance the reduced production of leucocytes caused by maturation arrest by keeping alive the aged cells. Klima (1938) denies the occurrence of this compensatory mechanism, but states that the process of hypersegmentation commences in immature cells. He observed indenta-



FIG 101 Bizarre "giant metamyelocytes" and giant neutrophils From the sternal marrow in pernicious anæmia (× 1,050)

tion of the nucleus and commencing segmentation in myelocytes, even before the cells had reached the necessary structural maturity With Jones (1937), Kienle and others we have confirmed this observation. We used in this connection the expression "dissociation of maturation in nuclear shape and structure," which is the exact opposite of Pelger's anomaly (p. 303). In pernicious anæmia the shape of the nucleus is old and the structure juvenile, in Pelger's anomaly the shape is young and the structure old In both Kienle's (1943) and our observations the tendency to hypersegmentation does not run parallel to the degree of anæmia He described some cases in which megaloblastic transformation of the marrow, and others in which changes in the granular cells predominated, and therefore believed that the constitutional element played a large part Enlargement and hypersegmentation of the granular cells occur in all cases of true pernicious anæmia in our experience In some cases the giant neutrophils are particularly numerous (Fig 101)

In the sternal marrow the white cells return to normal somewhat more slowly than the red cells. This return could be recognized in all our cases within a week of the commencement of treatment. In case 1 (p 75) the *myeloid shift to the left was quite definite* in the first sternal puncture: 4·5% promyelocytes, 6 5% semimature, and 14% mature myelocytes In the third puncture figures had returned to normal, and at the same time the tendency to hypersegmentation regressed.

Disturbance of thrombocytopoiesis in pernicious anæmia can on occasions reach such a degree that it may cause purpura and hæmorrhages In 1935 we reported a case with hæmoptysis, which prior to admission to hospital was diagnosed as active pulmonary tuberculosis. In case 1 (p. 75) there were severe attacks of epistaxis, but when the number of platelets had risen after liver



Fig. 102



Fig 103

Fig 102. Hypersegmented megakaryocyte in sternal marrow in pernicious anæmia (× 1,000)

Fig 103 Hypersegmented megakaryoblast in promegaloblast-megaloblastic marrow in pernicious anæmia (× 1,500)

therapy from 9,800 to 282,000 in 14 days, no further bleedings occurred Arneth (1942) states that a shift to the right of the qualitative platelet picture is characteristic In pernicious anæmia we have found that the megakaryocytes in sternal marrow are scanty and often show some hypersegmentation, enlargement and evidence of degenerative changes (Fig. 102) Similar findings have been recorded by Tempka and Braun (1932) Dameshek and Valentine (1937), Henning and Keilhack (1939), Rohr (1940), Kienle (1943), Piechl 1943), and Thaddea (1943) and in post-mortem material by Askanazy (1927) and Frey (1928) An increase in marrow giant cells has been reported by Heilmeyer (1938) (one case only) and by Kienle (1943) but we have never observed such an increase Hypersegmentation of these giant cells may commence sometimes in the megakaryoblasts (Fig 103) (Thaddea, 1943), but Kienle found hypersegmentation to begin only in the promegakaryocytes. Tempka and Braun (1932) noted numbers of isolated nuclei of megakaryocytes, but the deficiency of platelet-forming marrow giant cells is the most striking

feature in our experience. The return to normal of the marrow giant cell picture with liver therapy is an even slower process than that of the white cells, and in our cases did not occur until the second to fourth week of treatment. According to Kienle it may be deferred for up to 6 months, without, however, resulting in significant thrombocytopenia.

Japa (1945) considers that in pernicious anæmia the essential abnormality of hæmopoiesis, which affects all three systems, is the inhibition of the capacity for cell division, resulting in prolonged duration of mitosis, their decreased frequency, and earlier cessation with consequent differentiation at an early genealogical stage. These changes seem to be related, he believes, to lack of proper development of the nucleoli and changes in nucleic acid metabolism.

As far as the marrow reticulum cells are concerned, Dameshek and Valentine (1937) record an increase of histiocytes; Nordenson (1938) an increase of Ferrata cells; Heilmeyer (1938) an increase and enlargement of the lymphoid reticulum cells; Rohr (1938) an increase and enlargement of the reticulo-endothelial cells, but Markoff (1937) failed to confirm this. Piechl (1943) in his studies of the separation of marrow cells from the marrow tissue noticed a large increase of reticulum cells. According to Tischendorf (1941) increased erythropoiesis is invariably accompanied by reticular hyperplasia, especially when as in pernicious anæmia the marrow is megaloblastic. The increase of reticulum cells persists even after the transformation of megaloblastic marrow following liver therapy. In 9 cases we found an increase in primitive and phagocytic reticulum cells, in 7 cases a slight increase in primitive reticulum cells and in only 4 cases were the reticulum cell counts normal. Reticulum cells in the marrow show a much slower return to normal than the red cells, and the leucocytes With the exception of one case, all returned to normal within 4 weeks. Schleicher (1945) reports the marrow findings in 102 cases of pernicious anæmia He believes that the fundamental cause of pernicious anæmia is disease of the marrow reticulum and gives details of the morphological changes present in the cells in relapse and as treatment progresses. We agree with Klima (1938) that the plasma cells are increased and this has been confirmed recently by Kienle (1943)

Only a few of the other hæmatological findings in pernicious anæmia need be mentioned here: Lombardi (1928), Cassano (1930, 1940), and Moeschlin (1942) observed increased fragility of erythrocytes Bilirubinæmia is a sign of blood destruction, and so is the increased excretion of katabolic products of the blood. Urobilin, urobilinogen, uroerythrin and other pigments belonging to the urochrome group are present in the urine, but Bingold's pentdyopent is absent, because the capacity of the kidneys to oxidise bilirubin has been lost. Pentdyopent is a product of blood katabolism, which on spectroscopy occupies a band at 525 A U The name was derived

by Bingold (1932) from the Greek numerals for 5, 2 and 5 Stercobilin is excreted in the fæces, and bilirubin in the bile, both in increased amounts. The increased excretion of Porphyrin I and III in the urine, found by Duesberg (1931), Brugsch (1936), Vannotti (1937), Watson(1938) and Cotti (1940) indicates not only an increased destruction of erythrocytes, but also a disordered synthesis of hæmoglobin. Seggel (1940) found that the erythrocytes in pernicious anæmia contained an increased amount of porphyrin. It is probable that porphyrin production is particularly increased when the iron stores of the body have been exhausted. The anabolic processes are reduced in untreated pernicious anæmia when compared with the katabolic processes. Vandenbroucke (1941) found the antihæmolytic quality of the serum, as measured by the inhibition of the hæmolysis of lysolecithin, reduced. Heilmeyer and Plötner (1937), Skouge (1939), Büchmann (1941), Waldenström (1944), and Broehner-Mortensen (1943) found the serum-iron was raised, but that it often became lowered with liver therapy.

In megalocytic anæmias hypocholesterolæmia (in our case 86 mg.%) has been observed. Markoff (1942) records values between 47 mg.% and 110 mg %, instead of the normal 160 mg.%. Similar findings have been obtained by Vallois and Carrera (1935) and Chatterjee (1940) in pernicious anæmia of pregnancy, and they have used cholesterol prophylactically with success. This, however, is certainly not therapy on ætiological grounds, but rather one to spare erythrocytes by peripheral action. According to Brindeau (1934) cholesterol has an anti-hæmolytic action, and this is an additional advantage

The cause of pernicious anæmia will be discussed here only in so far as it relates to the question of what types of pathological blood pictures should be included in the pernicious group The disturbance of erythroblastic maturation depends, according to Castle, on the deficiency of the anti-pernicious principle, which we know since Minot's and Murphy's discovery in 1926 is contained in liver. It is formed from Castle's "extrinsic factor," which is taken in the diet, especially in meat, eggs, yeast and certain cereals, and which is not a protein body, but is thermostable (Reimann, Hemmrich and Steiner, 1936, and others) By the action of Castle's "intrinsic factor" on the extrinsic factor, the anti-pernicious principle is formed and is stored in the liver Meulengracht (1935) has shown that in hogs the intrinsic factor is produced mainly in the pyloric region of the stomach Henning and Keilhack (1936) and Deelman (1940) think it is produced in the cardiac region According to Kuhnau (1933) it is contained also in the duodenum, according to Schemensky (1935) in the colon, and according to Tempka (1937) even in saliva Fox and Castle (1942), while confirming Meulengracht's work on pigs, have shown that in man the cardiac end of the stomach is the site of the formation of the intrinsic

factor. Castle, Heath, Strauss and Heinle (1938) believe the intrinsic factor to be a proteolytic enzyme. After removal of pepsin and trypsin at *p*H 6 0 it may be demonstrated in the gastric juice by Lasch's (1938) method or by Singer's (1935) reticulocyte reaction in rats. The anti-pernicious principle is a labile, water soluble substance of so far unknown chemical composition, and it cannot be produced in the absence of either the extrinsic or the intrinsic factors. That it is a very complex substance is suggested by the work of Jacobson and Subbarow (1937).

The question arises whether all anæmias in which the absence of Castle's factors leads to a megalocytic anæmia of Addisonian type, should be regarded as one and the same disease, or whether we should differentiate between true pernicious and pernicious-like anæmias. In the idiopathic form the anæmia results from the absence of the intrinsic factor in the gastric juice; in sprue from faulty absorption owing to disease of the intestine; in hepatic disease from the impossibility of storing the anti-pernicious principle owing to reduction in liver parenchyma (only once observed by us), and in toxic conditions such as pregnancy, infestation with diphyllobothrium latum and goat's milk anæmia from increased demand for the principle. However, in all these various anæmias the deficiency of anti-pernicious principle is the causal factor in the production of the characteristic form of anæmia. Rohr (1940) has therefore classified them as follows :—

(1) Idiopathic pernicious anæmia of gastric origin

(2) Pernicious anæmia of sprue from failure of absorption

(3) Pernicious anæmia in liver disease from inability to store

(4) Pernicious anæmia in toxic conditions from exhaustion of the reserves of anti-pernicious principle.

(5) Pernicious anæmia from vitamin deficiency

This last type added by us has been observed especially in the tropics in malnutrition, especially Vitamin B_2 deficiency. The extrinsic factor of Castle is usually absent in this condition. All these anæmias show megalocytes in the peripheral blood and megaloblasts in the marrow, and all are hyperchromic, although of course if there is super-added iron deficiency they may become hypochromic It is useless to discuss whether the term "pernicious" is properly applicable or not This name is merely traditional, and as such it is useful though no longer true, since liver treatment has made this anæmia one of the most easily corrected diseases It would be better to call it Addison's or Biermer's anæmia. The collection of these megalocytic anæmias into one class is not universally recognized Clinically, as well as hæmatologically, there are certain distinguishing features, though this does not alter the fact, that all these anæmias are actually deficiency diseases of the unknown vitamin-like factor, the anti-pernicious principle

Apart from a hereditary disposition (Alder, 1939; Kaufmann and Thiessen, 1941; Schemm, 1940; Stamos, 1940; Thiele, 1938; Werner, 1938), chronic gastritis plays an important part in idiopathic pernicious anæmia (Dennig, 1929, 1939; Velde, 1934; Thiele, 1938; Kalk, 1939) Schindler and Serby (1939) have observed chronic gastritis and achylia in 23 cases of pernicious anæmia on gastroscopy. Haring (1932), however, disputed the importance of chronic gastritis Erkelentz (1935), Dennig (1939), Kalk (1939), Thaddea and Sauerbruch (1939) and Cotti (1940), and others believe that both carcinoma of the stomach, often observed in pernicious anæmia, and pernicious anæmia itself are due to chronic gastritis Owing to the general prolongation of life we are now able to observe pathological features which previously had been unknown. Fiessinger, Albeaux-Fernet and Lajouanine (1941) collected 50 cases of carcinoma of the stomach and pernicious anæmia during the last 10 years, and came to the conclusion that pernicious anæmia favoured the development of carcinoma of the stomach, while other forms of cancer are extremely rare in pernicious anæmia (*cf.* Steinbrinck, 1941) We believe that the gastritis, not the anæmia, is the causal factor of the carcinoma.

Monasterio (1939) found that of 40 cases with resection of extensive areas of gastric mucosa for ulcers or cancers, 7 developed megaloblastic anæmias Buchgraber and Fleischhacker (1938) found this in 13 of 27 cases, and other authors who have investigated this question are Rowlands and Simpson (1932), Richter, Ivy and Meyer (1933), Sturgis and Goldhamer (1939), Thaddea (1940) and Umber (1942). It may therefore be assumed that extensive damage of the gastric mucosa from chronic inflammation leads to an elimination of certain functions, which may to all intents and purposes equal the effects of resection. It is, of course, only natural that not every case of chronic gastritis or gastrectomy will develop megaloblastic anæmia, as small areas of gastric mucosa which may be preserved intact are able to produce sufficient intrinsic factor The factor appears to be more resistant than the secretion of pepsin or hydrochloric acid It has been demonstrated even in absolute achlorhydria Margottini (1940) did not find a single case of pernicious anæmia in 84 patients who had a gastrectomy It appears that iron-deficiency anæmia is more common among these "agastric anæmias" (Morawitz, 1930) and women are affected more frequently than men (Morawitz, 1930, Meulengracht, 1933, Hartfall, 1934; Larsen, 1934, Alder, 1937; Dreher, 1938, Heilmeyer, 1938; Leroux and Vermes, 1939, Murányi, 1940) In the production of agastric anæmias constitutional factors may possibly play a part.

Summary. The sternal marrow in pernicious anæmia shows very striking changes which are of value in diagnosis and prognosis —

factor. Castle, Heath, Strauss and Heinle (1938) believe the intrinsic factor to be a proteolytic enzyme. After removal of pepsin and trypsin at *p*H 6 0 it may be demonstrated in the gastric juice by Lasch's (1938) method or by Singer's (1935) reticulocyte reaction in rats. The anti-pernicious principle is a labile, water soluble substance of so far unknown chemical composition, and it cannot be produced in the absence of either the extrinsic or the intrinsic factors. That it is a very complex substance is suggested by the work of Jacobson and Subbarow (1937).

The question arises whether all anæmias in which the absence of Castle's factors leads to a megalocytic anæmia of Addisonian type, should be regarded as one and the same disease, or whether we should differentiate between true pernicious and pernicious-like anæmias. In the idiopathic form the anæmia results from the absence of the intrinsic factor in the gastric juice ; in sprue from faulty absorption owing to disease of the intestine ; in hepatic disease from the impossibility of storing the anti-pernicious principle owing to reduction in liver parenchyma (only once observed by us), and in toxic conditions such as pregnancy, infestation with diphyllobothrium latum and goat's milk anæmia from increased demand for the principle. However, in all these various anæmias the deficiency of anti-pernicious principle is the causal factor in the production of the characteristic form of anæmia. Rohr (1940) has therefore classified them as follows —

(1) Idiopathic pernicious anæmia of gastric origin.

(2) Pernicious anæmia of sprue from failure of absorption.

(3) Pernicious anæmia in liver disease from inability to store.

(4) Pernicious anæmia in toxic conditions from exhaustion of the reserves of anti-pernicious principle

(5) Pernicious anæmia from vitamin deficiency.

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Summary. The sternal marrow in pernicious anæmia shows very striking changes which are of value in diagnosis and prognosis —

(1) Great cellularity (excepting rare cases, which are poorly cellular).

(2) Presence of megaloblasts.

(3) *Disturbance of myelocytic maturation with large neutrophils* and hypersegmentation of primitive and senile forms (probably also degenerative changes)

(4) Diminution in megakaryocytes with hypersegmentation and degenerative changes.

(5) Hyperplasia of reticulum cells and plasma cells (inconstant) We are, therefore, dealing not exclusively with a disorder of erythropoiesis, but with an affection of all blood cell systems, a panmyelopathy, in which the damage to the red cells is *merely the most* obvious characteristic. During a relapse the marrow becomes megaloblastic only when liver therapy has been suspended for a long time, *sometimes a matter of years. After a shorter interval relapsing cases* often only show a rather cellular marrow with scanty megaloblasts and numerous basophilic normoblasts.

Megaloblastic Anæmia of Sprue

Megaloblastic anæmia is a frequent though not regular feature of sprue, although it is not uncommon. The earliest observation was made by Krjukoff (1931). Larger series have been reported by Rhoads and Castle (1933) (19 cases), Vogel, Erf and Rosenthal (1937) (5 cases), Markoff (1938) (10 of 17 cases), Hotz and Rohr (1939) (13 of 20 cases). Hansen and Staa (1936) (whose case came to autopsy), Merwe (1936), Schulten (1939) each in a single case failed to find megaloblasts Mackie and Fairley (1929) *in a series of 8 cases examined post-mortem found a hyperplastic* megalo-normoblastic marrow on only two occasions, the other cases showing merely marrow aplasia. The pitfalls of observations on bone marrow at post-mortem *have* been *already reviewed (p 22)* Only in a certain number of cases of sprue are disturbances of absorption severe enough to produce a megaloblastic anæmia.

Such disturbances may result in megalocytic anæmia in other disease states also In cœliac disease (Gee's disease, intestinal infantilism, infantile sprue), megalocytic anæmia has been observed by Fanconi (1937), and megaloblastic marrow reaction by Vischer (1938). Faber (1913), Strandell (1931), Hawksley and Meulengracht (1936), Barker and Hummel (1939) and Brock (1939) have observed pernicious anæmia in strictures of the colon, Plum and Warburg (1939) in regional ileitis, and other authors in cases who had intestinal resections It may be argued, as for example by Schairer (1940), that strictures of the colon develop following an ulcerative process in the intestine as has sometimes been observed in pernicious anæmia *Certain observations have, however,* been made which have proved the great importance of disturbances of absorption Fairley and

Kilner (1931) reported a case of gastro-ileo-colic fistulæ and sprue-like stools, in which the megalocytic anæmia was completely cured after excision of the fistula Sturgis and Goldhamer (1939) have seen similar cases. The importance of the intestinal canal in the pathogenesis of sprue, in opposition to the old theory implicating the suprarenals, has been confirmed by hæmatological observations of the anæmias in sprue.

There are certain hæmatological differences between the idiopathic and the sprue forms of pernicious anæmia. Marked karyorrhexis is usually found in sprue, but is only seen in agonal states in the idiopathic form. In the peripheral blood uniformly large megalocytes are seen (isomegalocytosis), whereas Addisonian anæmia is characterized by anisocytosis and poikilocytosis. Rohr (1936) believes that these phenomena in pernicious anæmias are due to hyperfunction of the spleen, which causes increased destruction of the rather fragile megalocytes. Owing to this increased destruction, immature hastily-formed poikilocytes reach the blood stream. On the other hand, in sprue there is atrophy and therefore hypofunction of the spleen, with many Howell-Jolly bodies in the blood, described by Hirschfeld and Dunner (1933) as reminiscent of observations on patients after splenectomy Evidence of increased blood destruction and bilirubinæmia are also absent or only slight in sprue while the intrinsic factor is usually present in the gastric juice (Barnett, 1931, 1932; Castle and Rhoads, 1932; Markoff, 1938) The return to normal of a previously achylic gastric juice following liver therapy excludes idiopathic pernicious anæmia and favours a diagnosis of anæmia of sprue according to Rohr (1936) The rather slower action of liver treatment in anæmia due to strictures of the colon and other anæmias due to lack of absorption (Butt and Watkins, 1936) does not in our opinion constitute a sufficiently definite difference from Addison's anæmia. The action of liver in pernicious anæmia of sprue is not always sluggish as is shown by the following case :—

Case 2. W. T , a woman of 70 years, had diarrhœa for 8 years Family [illegible]

gestion of glossitis Dyspnœa and palpitations on slightest exertion. Blood pressure 100/100 mm Hg Heart. apical systolic murmur. Lungs normal Liver edge percussed at costal margin Spleen enlarged as judged by percussion, almost reached costal margin. Blood picture (see table 5) indicated pernicious anæmia Therefore treatment with Campolon started and diet rich in vitamins ordered

STERNAL MARROW, obtained by puncture one day after an injection of 5 c c Campolon, showed by then normoblastic hyperplasia, but megaloblasts were still present in large numbers Myelogram · proerythroblasts 8, early normoblasts 20, normoblasts 300, megaloblasts 17 per 100 white cells,

myeloblasts 1·3%, promyelocytes 4·6%, semimature myelocytes 8 6%, mature myelocytes 16·3%, metamyelocytes 19%, stabforms 17%, segmented polymorphs 7·3%, basophil myelocytes 0·6%, basophils 0 3%, eosinophil myelocytes 3%, eosinophil metamyelocytes 2·6%, eosinophils 4%, lymphocytes 2%, monocytes 1%, megakaryoblasts 0 6%, megakaryocyt[illegible] plasma cells 0 6%, lymphoid reticulum [illegible] cells 1%, fat cells 0·3%. Blood pictu[illegible] ing Campolon and Pernaemon forte treatment).

TABLE 5 (*pernicious anæmia of sprue*)

Date	1942 13 5	19 5	24 5	30 5	2 6	6 6	10 6	21 6
Hæmoglobin (100% = 16 g %)	30%	38%	50%	61%	58%	68%	64%	78%
Erythrocytes . . millions per cmm	1·16	1 54	2 46	2 54	3 12	3 6	3 452	3·92
Colour index .	1 36	1 23	0 98	1·17	0 93	0 95	0 99	0 97
Reticulocytes . .	1·2%	23%	7 0%	3 4%	3 0%	2 3%	—	2 6%
Megaloblasts .	3 0%	1 0%						
Normoblasts	3 0%	2 0%						
Aniso-poikilocytosis .	+ + +	+ +	+	(+)				
Platelets per cmm .	55,000	120,000	200,000	285,000	286,000			305,000
W.B C. per cmm. .	2,750	3,680	5,460	5,360	5,420	4,800	4.500	6,200
Basophils %	1 0			0 5	1 0		1 5	
Eosinophils %	5 0	7 0	7 5	6 5	8 0	10 0	11 5	3 5
Stab forms % .								0 5
Segmented polymorphs %	24 0	26 0	60 5	60 5	52 0	45 0	40 0	71·0
Lymphocytes % .	67 0	55 5	23 5	23 0	34 0	39 0	42 0	17 0
Monocytes % .	3 0	10 5	8 5	5 0	5 0	6 0	4 0	8 0
Plasma cells% .				1 5				

Hæmatologically everything pointed to pernicious anæmia: severe anæmia with megalocytes, leucopenia, typical hypersegmented forms, thrombocytopenia, relative lymphocytosis Though anisocytosis and poikilocytosis were not as pronounced as is usual in Addisonian anæmia, isomegalocytosis was not present. In any case a diagnosis of sprue was not justifiable on the grounds of isomegalocytosis alone Typical megaloblasts (Fig 104) and giant neutrophils (Fig 105) were found in the marrow A slight deterioration occurred on June 10th, 1942, Ferro-Redoxon was administered, and the number of erythrocytes increased, as also did the hæmoglobin. The reduction of granulocytes between June 2nd, 1942, and June 10th, 1942, is interesting as well as their increase on June 21st, 1942, when the lymphocytes decreased The administration of iron might possibly have stimulated the hæmopoietic reticulum to produce more granulocytes

Rodriguez-Molina (1941), who reported 100 cases of anæmia of sprue, often found giant neutrophils In our case morphological evidence of damage to the megakaryocytes could not be established Anisocytosis and poikilocytosis was not so marked as in true pernicious anæmia Howell-Jolly bodies (Fig. 106) were often

present, but other findings would not have permitted differentiation from Addisonian anæmia.



Fig. 104.



Fig. 105.

Fig. 104 Mitosis of a megaloblast in sternal marrow in pernicious anæmia of sprue. (× 1,400)

Fig. 105 Giant neutrophils in sternal marrow in pernicious anæmia of sprue. (× 1,400)

Broehner-Mortensen (1943) found in 2 cases of megalocytic anæmia of sprue a low serum iron level in contrast to the findings in idiopathic pernicious anæmia In an attempt at iron-saturation the injected iron rapidly disappeared from the blood stream.

Summary. (1) In sprue, megalocytic anæmia of the pernicious type is relatively common. (2) Sternal marrow is cellular with megaloblasts and giant neutrophils as in true Addisonian anæmia (3) Karyorrhexis and Howell-Jolly bodies are more common in megalocytic anæmia of sprue than in true Addisonian anæmia The blood picture often shows isomegalocytosis instead of anisocytosis and poikilocytosis (4) Further differentiation may be possible by investigation of the serum and of the gastric juice (no bilirubinæmia, no increase in serum iron) (5) The classing of anæmia of sprue with the anæmias of the pernicious type is justified as regards the effect of anti-pernicious principle in treatment



Fig 106 Trinuclear erythroblast with Howell-Jolly body in pernicious anæmia of sprue (× 500)

Toxic Pernicious Anæmia from Exhaustion of the Hæmopoietic Principle

Diphyllobothrium Anæmia. From Schauman's (1894) investigation it appears that in infestation with diphyllobothrium latum, megalocytic anæmias may develop, which are almost identical hæmatologically with true pernicious anæmia. This occurrence is in fact rather rare and Ehrström (1928) found only one case of megalocytic anæmia in 5,000 patients infected with the worm Constitutional and racial factors apparently play a large part. In Japan where diphyllobothrium infection is widespread, Kumagai

and Shimizu (1934) have rarely observed a case with megalocytic anæmia. In 50 infected patients Marujama and Tanaka (1940) found a mild anæmia in 23·5%, but no case of severe pernicious-like anæmia was seen Birkeland (1932) reported that Finlanders comprised 70% of 550 cases of diphyllobothrium anæmia whereas Swedes, Italians and Japanese, who are equally heavily infected, rarely showed anæmia.

Opinions vary about the ætiology of pernicious anæmia in *diphyllobothrium infestation*. Von Bonsdorff (1939) believes that the improvement of the anæmia following the expulsion of the worm is a strong point in favour of the worm being the causal factor. Such improvement, however, only occurs when the diet is sufficiently rich in the extrinsic factor. Von Bonsdorff maintains that the intrinsic factor remains unaffected. Hernberg (1941), by using Lasch's method, has found the intrinsic factor to be about one-third of the normal amount, and Heilmeyer (1942) believes that the reappearance of free hydrochloric acid in the gastric juice, reported by Becker (1931) and Hoff and Sauerstein (1936) after expulsion of the worm, suggests a diminution of the intrinsic factor. Von Bonsdorff believes that the worm contains some water-soluble, thermostable substance, which may be precipitated by alcohol This substance is alleged to inhibit the hydrolysing faculty of the intrinsic factor, which in his opinion is a proteolytic enzyme Töttermann (1938), by using an alcoholic extract of dried worm substance and administering it to patients who had had diphyllobothrium anæmia, was able to produce a fall in erythrocytes and a rise in the colour index He therefore deduces that these processes are due to some *hypersensitivity mechanism*. Töttermann (1939) has examined sternal marrows in diphyllobothrium anæmia, and in 111 patients with worms he found 12 cases with megaloblasts and promegaloblasts in the marrow. This observation also confirms the occurrence of pernicious anæmia in persons infested with diphyllobothrium latum The therapeutic success of liver extracts, just as in the megalocytic anæmia of sprue, may be interpreted similarly

Pernicious Anæmia of Pregnancy. Pernicious anæmia of pregnancy has been recognized since the papers of Naegeli (1931) and his assistants, Beyer-Gurowitsch (1912) and Filo (1931) Strauss and Castle (1932) observed a diminished production of the intrinsic factor in the stomach, frequently with achlorhydria. During pregnancy the presence of the fœtus causes an increased demand for the hæmopoietic principle. Constitutional predisposition may play a part Pontano (1912), Henning (1935), Schulten (1939) and Rohr (1940), have reported cases in which pernicious anæmia recurred during successive pregnancies. Pernicious anæmia of pregnancy is a very rare disorder According to Abramson (1938) and Callender (1944) distinction cannot be made by bone marrow examination from the idiopathic form of pernicious anæmia

Segerdahl (1941) found only scanty megaloblasts in sternal marrow, and achlorhydria was not a constant feature Miller and Studdert (1942) found free hydrochloric acid in the gastric juice in 18 of 23 cases. If the pernicious anæmia does not remit after the termination of pregnancy, a combination of pregnancy with true Addisonian anæmia must be presumed. Alder (1939) and Hüssy (1940) are of the opinion that a latent pernicious anæmia may become activated by pregnancy. A case reported by Stodtmeister and Büchmann (1942) illustrates the great care necessary in diagnosis; the marrow showed red cell hypoplasia, but cells resembling megaloblasts were present. The authors finally excluded pernicious anæmia as a diagnosis on the basis of the subsequent course, and decided it was a case of aplastic anæmia. Davidson, Davis and Innes (1942) report 16 cases of severe megaloblastic anæmia during pregnancy, in which the diagnosis was based on sternal puncture. The colour index was 1 0 or less, quite unlike Addisonian anæmia Of 14 patients examined, 10 had free hydrochloric acid in the gastric juice As liver preparations did not succeed until supplemented by blood transfusion, it is doubtful if these cases can be regarded as pernicious anæmia of pregnancy, although the response to liver extract in pernicious anæmia of pregnancy is usually slower than in true pernicious anæmia Damachij (1936) alleges that in 10 apparently normal expectant mothers examined by sternal marrow biopsy, he has always found megaloblasts This statement is open to criticism, and can only be explained by an unorthodox definition of the megaloblast We have never seen megaloblasts in normal pregnancy Callender (1946) examined the marrow of 19 healthy women in pregnancy and the puerperium and found no megaloblasts, but a slight hyperplasia only in the late weeks of pregnancy and the early days of the puerperium Hæmatologically, pernicious anæmia of pregnancy is almost indistinguishable from Addisonian anæmia, and its diagnosis in a severe anæmia of pregnancy is only assured by actually finding megaloblasts in the marrow (Heilbrun, 1936, Markoff, 1939, Hussy, 1940, Rohr, 1940; Guggisberg, 1941, Thaddea, 1943)

Pernicious Anæmia from Failure of Storage

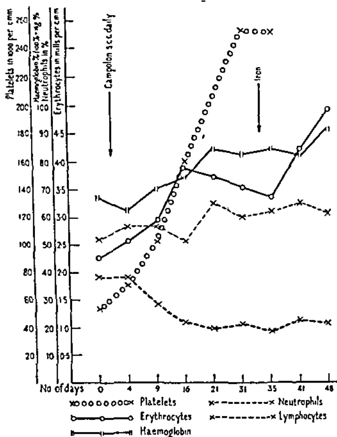
This type of pernicious anæmia occurs in patients with hepatic disease, which may prevent storage of the anti-pernicious principle Goldhamer Isaacs and Sturgis (1934) have in fact proved in a case with cirrhosis and a megalocytic anæmia that the liver contained no hæmopoietic principle, but it has been present in other cases (Schiff, Rich and Simon 1938) Davidson and Fullerton (1938) suggest that the megalocytic anæmias of liver disease are caused by failure of the final stages of synthesis of the hæmopoietic principle, rather than a failure of storage Cases of pernicious anæmia in patients

with hepatic disease are rare. We have seen megaloblasts in the sternal marrow of patients with cirrhosis of the liver in only one case. Similar observations were made by Schulten (1933), Markoff (1938) and Klima (1940) Hotz (1941) has collected 22 cases. They are distinguished from the idiopathic form of pernicious anæmia by the frequently present free hydrochloric acid in the gastric juice (Wintrobe, 1936), by the macrocytosis, the absence of a relative lymphocytosis and by the rather higher figures of reticulocytes *prior to liver therapy*. Liver preparations are efficacious, though after recovery macrocytosis remains Rossier (1932), Rohr (1940), Hotz (1941), Leitner (1941) and Thaddea (1943) found megaloblasts in the sternal marrow.

Other Pernicious and Pernicious-like Anæmias

Infectious diseases, notably syphilis (Naegeli, 1931), tuberculosis (Bykowa, 1927; Schneiderbaur, 1938), malaria (Toullec and *Jolly, 1931; Schretzenmayr, 1938; Ghizetti, 1939), and B. coli* sepsis (Nanu-Muscel, Jonnesco and Valter, 1931) have been stated to produce a pernicious anæmia According to Heilmeyer (1942) only one single case of syphilis of those reported in the literature will withstand criticism. The case of sepsis reported by Nanu-Muscel, Jonnesco and Valter (1931) is certainly not pernicious anæmia but a toxic hæmolytic anæmia. Though it is theoretically possible that a chronic infectious disease and chronic gastritis associated with it may result in a decrease of the intrinsic factor (Thiele, 1938), we believe this to be an extraordinarily rare occurrence. During the last 17 years' work we have never met a single case of pernicious anæmia which might have been caused by tuberculosis, or any other infectious disease, though combinations of pernicious anæmia and tuberculosis have been observed. Thus the ætiological importance of infectious disease in the causation of [illegible] anæmia must be viewed with extreme scepticism [illegible] (1938) and *Bianchi (1940) have reported megalo-*[illegible] in malaria. The combination of infectious na[illegible] anæmia is of hæmatological interest. The his [illegible] literature show how difficult it can be to arrive and Ca[illegible] According to Thaddea and Sauerbruch factor in t[illegible] may become hypochromic under the nancy the p[illegible] when the case has had treatment the hæmopoie[illegible] (1934), when polyarthritis co-play a part Po[illegible] from the marrow. Markoff and Rohr (1940), ha[illegible] [illegible]erculosis, and recurred during succ[illegible] (1939) [illegible] en it happen pregnancy *is a very rare* [illegible] "dis-and Callender (1944) distin[illegible] ow-examination from the idiop[illegible]

Case 3. G E., a woman of 46, with a negative family history, had had measles, mumps and rheumatism In 1933 she was admitted to hospital with pernicious anæmia, complaining of backache November, 1938, considerable pain in hips and back On admission to hospital, lumbar spondylitis was found and a pernicious-like anæmia, which improved with blood transfusions and liver therapy. When admitted the 1st, 2nd and 3rd lumbar vertebræ were found to be affected by tuberculosis, which was treated by a plaster bed, ultra-violet light and mercury vapour lamp. December, 1939, anæmia recurred.



GRAPH 6 Pernicious anæmia and tuberculous spondylitis with anæmia, leucopenia, thrombocytopenia Return to normal of blood levels after Campolon and iron therapy

BLOOD R B C 2 3 millions, Hb 68% = 11 0 g %, colour index 1 47, W B C 2,280 per cm , polymorphs 52 5%, lymphoc 0 5% Hypersegmentation c cytosis, polychromasia, one normoblast Thrombocytopenia of 55,000, bleeding time 2 minutes, clotting time 6½ minutes. Sedimentation rate (Westergren) 60 mm in 1 hour

STERNAL MARROW Cellular with proerythroblasts 5 3, early normoblasts 14, normoblasts 58 3, megaloblasts 5 6 per 100 white cells,

myeloblasts 0 6%,
cytes 13%, stab fc
0 3%, eosinophil m
monocytes 4%, m
phagocytic reticulum cells 1 3%, plasma cells 1·3%. A sternal puncture, performed nine months previously (obviously during the course of liver therapy), showed no megaloblasts, only 2 3 proerythroblasts, 5 3 early normoblasts, and 36·5 *normoblasts*. *Fractional test meal:* achlorhydria, refractory to histamine. Course and progress are shown in Graph 6.

In spite of comparatively scanty *megaloblasts in the sternal* marrow we must regard this as a case of pernicious anæmia with megalocytosis, poikilocytosis, leucopenia with relative lymphocytosis, and thrombocytopenia, and with the corresponding marrow changes, *e g.*, myeloid shift to the left, giant neutrophils, cellularity and megaloblasts. The relatively low number of megaloblasts is probably due, first of all, to the infection, as liver therapy had been given so long ago (6 years and 1½ years respectively) *Following liver therapy with Campolon and Heparglandol forte* the anæmia improved, the number of platelets rose from 55,000 to 254,000, leucocytes from 2,880 to 6,320. When the hæmoglobin had risen to 83% = 13·3 g %, the anæmia failed to show any further improvement, but instead slightly deteriorated. This was eventually overcome by the administration of iron, and the hæmoglobin soon after rose to 100% = 16 g %. This instructive case illustrates the difficulties in the diagnosis of pernicious anæmia when complicated *by infection, and further teaches us to be cautious in the interpretation* of infection as being the causal factor of anæmia In this case the history and the several previous hospital admissions proved that pernicious anæmia had existed before the onset of tuberculosis. The question, whether pernicious anæmia and active tuberculosis are mutually exclusive, has been discussed elsewhere (Leitner, 1935) It is certain that in obvious pernicious anæmia concurrent active tuberculosis is rare, because the oxyphilic tubercle bacilli meet with unfavourable conditions in blood and tissue, which is poor in oxygen *Once anæmia has been compensated by liver* therapy this inhibition ceases, and tuberculosis may develop

Megalocytic Deficiency Anæmias

György (1934) has cured goat's milk anæmia with Cenovis (a Vitamin B preparation) after he had failed with liver preparations It is noteworthy that goat's milk is rich in Vitamin B_2 and yet anæmia from deficiency of Vitamin B may result *Veeneklaas* (1941) has examined the sternal marrow in cases of goat's milk anæmia He found, just as in the megalocytic anæmias of gastric origin in infants from deficiency of fruit and vegetables in the diet, that both the blood and marrow showed the same picture as described in idiopathic pernicious anæmia, viz, megaloblasts, giant

neutrophils, hypersegmentation of neutrophils, anisocytosis, poikilocytosis. These cases, which cannot be distingui hæmatologically from pernicious anæmia, might be called pernic anæmia of vitamin deficiency in which apparently the extri factor is lacking. Tscheschc and Wolf (1936) found in the pter a therapeutic agent which can improve goat's milk anæmia, th it is inefficient in the treatment of pernicious anæmia. Addisonian anæmia cannot be cured by the extrinsic factor al though Jaeger (1943) has reported improvement with lactoflavin nicotinic acid. Pilgerstorfer (1942) and others have failed to con Jaeger's findings.

Tropical Megalocytic Anæmia

This disease was described by Wills and her colleagues (19 who attribute it to the absence of the extrinsic factor from the This has been refuted by Ungley (1933), Lassen and Lassen (19 and Napier (1938). Wills (1930) and Elsom and Sample (1937) observed tropical megalocytic anæmia especially in under-nouri pregnant women in India. Wills and Bilimoria (1932), and W Clutterbuck and Evans (1937) have experimentally produced anæmia in monkeys by giving a diet deficient in Vitamin B, and cured it by giving yeast preparations, such as Marmite. Wint (1939) demonstrated that yeast is rich in the extrinsic factor, therefore Wills's theory is not without basis Spies, Payne Chinn (1934) believe this anæmia to be macrocytic, *i e*, derived f early normoblasts and not from megaloblasts, and deny any relat ship to pernicious anæmia. Wills's theory is further strength because Fairley, Bromfield, Foy and Kondi (1938) have obser megaloblasts in the marrow of refugees from Asia Minor who suffering from megalocytic anæmia of alimentary origin spleen was not enlarged in these cases, and there was no exce bilirubin in the blood. In Europe, Groen and Snapper (1937) two similar cases One was cured by liver and the other by e meat and a diet rich in vitamins Meulengracht (1938) also repo a further case, where anæmia developed through insufficient Kumagai and Shimizu's (1934) cases, diagnosed as pernic anæmia of pregnancy, may in fact be tropical megalocytic anæ of Wills's type Leucopenia and thrombocytopenia were features of these cases, and just as in Wills's series, it is eas understand that deficient nutrition (deficiency in vitamins, e especially in pregnancy where increased demands naturally e can lead to manifest deficiency diseases. Benhamou (1 recommends extra Vitamin B in tropical anæmias Kemp (1 studied the effect of folic acid. In three cases of nutritional m locytic anæmia with megaloblastic hyperplasia in the bone mar he found that the marrow resumed a normoblastic pattern and

the peripheral blood showed a more rapid improvement than would have been expected if the patients had been treated with large doses of liver extract parenterally.

The anæmia of *Beri-beri*, according to Shimazono (1931), and Hahn and Whipple (1939), is not in fact a Vitamin B deficiency anæmia, but really a hypoproteinæmia. A certain number of anæmias from deficiency of meat in the diet, described by Marchal, Roualt and Deprez (1942) and based on observations of malnutrition in the war of 1939–1945, also belong to this group. In 27 cases they found only one case of anæmia of the pernicious type; in all others the anæmia was normochromic and the sternal marrow showed either hypoplasia or aplasia of the erythropoietic tissue.

Umber (1942) observed vitamin B_1 deficiency in cases of anæmia after gastrectomy. These anæmias were refractory to liver and iron, but promptly recovered on nicotinic acid. Apparently the body, after resection of an extensive portion of the gastric mucosa, loses the faculty of producing nicotinic acid. Pilgerstorfer (1942) has found lactoflavin to be beneficial in anæmias after gastrectomy. Vitamin B is of considerable importance in pernicious anæmia, especially in the treatment of neurological complications (Russell, 1936; Schultz, 1942, and others).

Another deficiency anæmia is the pernicious-like anæmia of *Pellagra*, in which Krjukoff (1931) found megaloblasts but no evidence of increased hæmolysis Bassi and Varo (1941) found only slight disturbances of maturation in 5 cases. Hwang, Kuo and Shih (1940) also report only slight secondary anæmia in pellagra without typical changes in the marrow. Ostiz-Picon (1941) has shown in 14 cases that the intrinsic factor is present in the gastric juice of patients with pellagra by Singer's reticulocyte reaction in rats.

Achrestic Anæmia

Even more controversial than the subject of Vitamin B deficiency anæmia, is the relationship of the so-called achrestic anæmia of Israëls and Wilkinson (1936, 1940) to pernicious anæmia. Achrestic anæmia is a refractory anæmia with large mean cell diameter of the red cells, in which the anti-pernicious principle, though present, is not utilized successfully Israëls and Wilkinson, and Benhamou (1938) found megaloblasts in the sternal marrow, but Adelheim (1930), Zanaty (1937) and Schulten (1939) could not confirm this

In our own opinion only those deficiency anæmias should be classed with pernicious anæmia which show the same hæmatological picture The points to be looked for are hyperchromia, megalocytosis, leucopenia with hypersegmentation, as well as thrombocytopenia in the peripheral blood, and a maturational disturbance of

the myeloid series in the marrow, as well as megaloblasts. On the other hand, it matters little whether one is dealing with a "gastric pernicious anæmia" (absence of the intrinsic factor), or "pernicious anæmia from failure of absorption" (inability to produce anti-pernicious principle), or "pernicious anæmia from vitamin deficiency" (absence of the extrinsic factor) or even with a toxic pernicious anæmia. Cases of macrocytic anæmia with free hydrochloric acid in the gastric juice should be classed as achrestic anæmia according to Israëls and Wilkinson, and Zanaty.

Recently we observed a case (Leitner, 1948), in which an idiopathic hypochromic anæmia was transformed into a typical pernicious anæmia with megalocytes in the peripheral blood and megaloblasts in the sternal marrow. Liver and Vitamin B preparations only resulted in very slight improvement There was a considerable increase of tissue mast cells in the sternal marrow, a phenomenon otherwise seen only in severe myelopathies. We therefore concluded that the disorder of hæmopoiesis in the various forms of anæmia is often of a more far-reaching character than appears at first sight. We believe that such cases as well as those with normal gastric function should be called achrestic. Deficient utilization of the liver principle appears to develop in these patients, whereas pernicious anæmia is usually of gastrogenic origin as shown by achylia and deficiency of the intrinsic factor. In the course of the disease extensive marrow damage occurs, which precludes the proper use of the anti-pernicious principle. So far it is not known whether the refractory behaviour of the marrow may be overcome by folic acid or other preparations. It is quite possible that here we have circumstances similar to those in the hypochromic anæmias of infectious or toxic origin, where anæmia will only respond after the active stage of the infectious disease or the toxic influence has been overcome Though iron deficiency may exist, iron preparations are useless until then. In megalocytic achrestic anæmia the underlying cause could be an infection or intoxication, which may remain undetected. In our case we suspected some focal infection

Summary: As a result of the work on the megaloblastic anæmias and especially on pernicious anæmia, sternal marrow biopsy is now firmly established as of great value in diagnosis, prognosis and control of therapeutic measures From an academic point of view, sternal puncture has provided many points for a fuller understanding of the mechanism of development of the various hyperchromic anæmias from deficiency, and for their classification.

HYPOCHROMIC ANÆMIAS

Idiopathic Hypochromic Anæmia

Classification of the hypochromic anæmias has been attempted only comparatively recently. Following the work of Einhorn

(1903), Faber (1909), and especially of Kaznelson, Reimann and Weiner in 1929, the "achlorhydric chlorotic anæmia" of Kaznelson was differentiated from the so-called secondary anæmias Schulten (1934), Bode and Heyrodt (1938) and others pointed out that achlorhydria is certainly not an invariable finding in this anæmia, though it is usual (Witts, 1930, 1931, Naegeli, 1931; Schinz, 1935; Weber and Huber, 1939, and others) Achlorhydria, which is refractory to histamine, is found in about 40% of cases; Lundholm (1939) found achlorhydria in 67%; and Witts (1930), Oliver and Wilkinson *(1933) and Hartfall (1934) in over 80% of* cases. Therefore Dameshek (1931) suggested the term "idiopathic hypochromic anæmia," and Schulten (1934) suggested "essential *hypochromic anæmia," to distinguish it from* symptomatic anæmias in infections, hæmorrhages, intoxications, tumours, etc. Further steps towards the problem of the classification of these anæmias were made by the observations of Mettier *et al.* (1933), Bethell *et al.* (1934), Heath and Patek (1937) and Davidson and Fullerton (1938) The first groups of authors suggested iron-deficiency as the causal factor of the anæmia, and this was confirmed by Heilmeyer and Plötner (1937). Faber (1913), Kaznelson *et al.* (1929), Leeuwen (1933), Schulten (1934), Heilmeyer (1938), Thiele (1938), Lundholm (1939) and others have worked out the symptomatology, which in many ways is similar to that of pernicious anæmia lassitude, paræsthesiæ (lesions of the spinal cord), loss of appetite, indigestion (sometimes from lack of acid in the gastric juice), dyspnœa, feeling of weakness, loss of muscle power (Schultz, 1935), glossitis, pharyngitis, dysphagia (Plummer-Vinson syndrome), cracks at the angle of the mouth, changes in the finger-nails (koilonychia), brittle hair which comes out easily (Dameshek, 1931), premature greying of hair, menorrhagia Some symptoms, such as koilonychia, are rarer in pernicious anæmia, or occur in a different form Plummer-Vinson syndrome, which according to Lundholm *(1939) occurs in 19% of all cases,* is due to atrophy of the mucosa of the hypopharynx and œsophagus, as observed by Suzman (1933) and Thiele and Kuhl (1938) McGee and Goodwin (1938) at *post-mortem found erosions in the mucosa* of the œsophagus and McGibbon (1935) and Allen (1941) found actual webs or bands across the œsophagus. Similar manifestations have been observed by *Waldenström (1938) in iron-deficiency even* without actual anæmia (cramp-like dysphagia) The suggestion by Suzman (1933), Singer (1934), Ahlbom (1936) Singer (1939), Waldenström and Kjellberg *(1939) that Plummer-Vinson syndrome is a* precancerous condition has not been confirmed by Laub (1938) and Weder (1943). Many of the symptoms in both pernicious anæmia and essential hypochromic anæmia *are due to the anæmia per se* rather than to the specific deficiency

Owing to the great similarity of the clinical manifestations

between the two types of anæmia, hæmatological findings assume the greatest importance for diagnosis. The low colour index, which is the most important sign, and from which the disease partly takes its name, is not always unequivocal. It is often only little less than unity, and indeed may be more. These latter cases, however, do not belong to the idiopathic hypochromic anæmia group A further diagnostic characteristic is the determination of the mean cell diameter of the erythrocytes. This may be done by halometry by Pijper's (1929) or Pryce's (1929) method, or by the methods of Schalm (1939) or Bock (1939). We have found it most reliable to measure the size of 100 cells and then to plot a Price-Jones curve In pernicious anæmia there is a shift to the right, that is to say an increase in size, but in idiopathic hypochromic anæmia the shift is to the left, that is to say a microcytosis, and a broadening of the base of the curve owing to anisocytosis (Heilmeyer, 1938) Probably the easiest and best method for clinical work for determining the cell size is by means of the hæmatocrit The type and method of Wintrobe (1933) is recommended By its use and with the knowledge of the red cell count and hæmoglobin, the mean corpuscular volume and mean corpuscular hæmoglobin concentration may be determined. In idiopathic hypochromic anæmia the mean corpuscular volume is almost always below 78 cubic microns (normal range 78–94) and the mean corpuscular hæmoglobin concentration is always below 32% (normal range 32%–38%). In other words this anæmia is typically microcytic and hypochromic Anisocytosis is also usually present. The thickness of the erythrocytes is reduced, the thickness index is smaller (Heilmeyer) The poor hæmoglobinization becomes obvious in the thin cells, especially in the centre where the cell collapses. In smears, therefore, the cells show a central white area bordered by a ring of colour (annulocytes, pessary forms) There is also a broadening of the red cell fragility curve with a tendency to increased resistance (Witts, 1930, 1932; Momigliano-Levi and Bairati, 1935; Daland and Worthley, 1935, Greppi, 1938).

The serum is pale in colour, which was first pointed out by Naegeli (1931) in cases of chlorosis Heilmeyer and Wappler (1928) have estimated the colour of the serum quantitatively by photometry. They found instead of the normal levels of 0·6–1·1 units, that there is a fall to 0·3 units owing to decrease of serum bilirubin The iron level in the blood is low Heilmeyer and Plötner (1937) found it between 20γ–56γ, Moore, Doan and Arrowsmith (1937) between 19γ and 46γ, Hoet, Vangoidsenhoven and Lederer (1939) 20γ–42γ, Skouge (1939) and other authors have also found low levels

The total number of leucocytes is not always reduced, but when it is, there is a granulocytopenia with a relative lymphocytosis just as in pernicious anæmia Leeuwen (1933), Schulten (1934)

and Leitner (1941) have also observed hypersegmentation of neutrophils.

Like Weiner and Kaznelson (1926), Dameshek (1931), Schulten (1934), Segerdahl (1934), Lundholm (1939), Rohr (1940), Kienle (1943), Thaddea (1943) and other authors, we found increased erythropoiesis in the marrow, which is mainly due to normoblasts, but proerythroblasts are also increased. We have never found megaloblasts and only rarely a significant increase in proerythroblasts, although the early basophilic normoblasts are almost always increased in numbers. Alder (1939) notes large erythroblasts in cases of iron-deficiency in chlorosis, while Markoff (1936) in chlorosis described a marrow with numerous large early normoblasts. Komiya (1938) found parallelism between Price-Jones curves on blood and marrow, and noted the presence of many micronormoblasts in the marrow. Weiner and Kaznelson (1926) found 75% erythroblasts, but Segerdahl (1935) only found an average of 28·59%. Reimann (1933) and Massobrio and Maranzana (1938) observed an increase in erythroblasts; Heilmeyer (1938) often found a predominance of basophilic normoblasts and proerythroblasts. Klima (1938) in one case found 8% proerythroblasts, and in a case of chlorosis 16% early and 38% late normoblasts. Thaddea (1943) in sternal marrow from a girl of fifteen with chlorosis observed great cellularity with many small basophilic erythroblasts. Following successful treatment with iron the figures for erythroblasts fell. According to Weiner and Kaznelson mitotic figures are reduced (0·67%), but both Segerdahl and ourselves found them increased; in one case 5·1% of the 25·5 erythroblasts per 100 white cells present showed mitoses. Markoff estimated them as high as 23%, but Fieschi (1940) found them almost normal at 1·6% Kienle (1943) states that the number of mitoses and the karyological curves are mostly normal, but there is a lag type of maturation curve *Sternal puncture often helps to corroborate the diagnosis of idiopathic hypochromic anæmia and sometimes helps to establish it.* The following case is quite typical:—

Case 4. A woman of 40 had felt tired for many years, katamenia normal October, 1937, normal childbirth December, 1937, increase of lassitude, difficulty in swallowing. On examination she was pale, apart from *koilonychia no other abnormal physical signs* Liver and spleen not enlarged

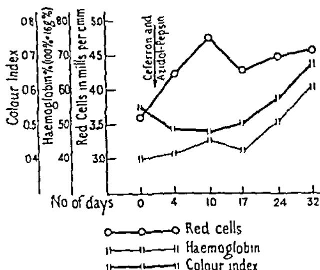
%, C I 0 55, W B C
%, segmented poly-
Blood sedimentation
l twenty-four hours

respectively) Anisocytosis; poikilocytosis, annulocytes Platelets 438,000 per cmm (Fonio's method). Reticulocytes 1·5% Bleeding time 1 minute 25 seconds (Duke's method) Clotting time 13 minutes 25 seconds (Bürker's method) Red cell fragility 0·52–0·36% NaCl. Test meal absolute achlorhydria refractory to histamine. Serum bilirubin direct negative, indirect 0 6 mg %, cholesterol 50 mg %

Takata-Ara reaction negative. Serum protein 9 12%, albumin/globulin = 50/50.

[illegible]

cells 0 3%. With iron her condition rapidly improved, as shown by Graph 7.



GRAPH 7. Idiopathic hypochromic anæmia with 3 65 million erythrocytes, hæmoglobin 40% = 6 4 g %, and a colour index of 0 55 Blood values returned to normal after Ceferron and Azidol Pepsin

The diagnosis in this case was based on the low colour index, which fell as low as 0 4, on the absence of megalocytes, on the absence of bilirubinæmia, absent leucopenia, absent thrombocytopenia, on anisomicrocytosis, and hypochromic annulocytes (see also Graph 8) Absence of megaloblasts and only moderate normoblastic hyperplasia in the sternal marrow served to confirm the diagnosis

The low grade normoblastic hyperplasia is often important for diagnosis, but so far has not received the attention which we believe it merits In the following case 5, in which the pathological picture had been distorted by previous treatment, it helped to establish the diagnosis definitely.

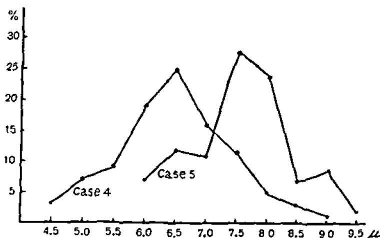
Case 5. G E , a 45-year-old woman, since the birth of a child became progressively weaker and paler Her doctors gave her iron as well as liver, without any success Hæmoglobin varied between 55-70% = 8 8-11·2 g %, but her general condition deteriorated steadily. Severe lassitude, loss of power, but no evidence of spinal cord lesions, no glossitis, no changes in the nails, no dysphagia, no splenomegaly.

BLOOD R B C 4 2 millions, Hb 77 7% = 12 4 g %. C I. 0 8, W B C 3,840 , basophils 2%, eosinophils 4%, stab forms 2%, segmented polymorphs 51%, lymphocytes 38%, monocytes 3%. Price-Jones

e of curve broadened. Sedimentation
e and two hours). Serum bilirubin
terol 160 mg.%; calcium 10·4 mg.%;

STERNAL MARROW. Proerythroblasts 0·6, early normoblasts 3, normoblasts 25·3 per 100 white cells; myeloblasts 2%, promyelocytes 3·3%, semimature myelocytes 4%, mature myelocytes 8·3%, metamyelocytes 10·3%, stab forms 10%, segmented polymorphs 26%, eosinophil myelocytes 1·3%, eosinophil metamyelocytes 1·6%, eosinophils 1·3%, lymphocytes 21%, monocytes 1·3%, megakaryocytes 0·6%, endothelial cells 1·6%, plasmoblasts 0·6%, proplasmocytes 1·3%, plasma cells 1%.

Following
dimentation



GRAPH 8. Price Jones curves in idiopathic hypochromic anæmia (Case 4, untreated, case 5, treated.)

This case shows well how difficult diagnosis can become, especially when a case has received previous treatment. The absence of megaloblasts could not be due to previous treatment, since she had had no liver for several years. The myelogram therefore favours idiopathic hypochromic anæmia, because in pernicious anæmia we should have expected a more pronounced erythroblastic reaction and also megaloblasts. The sternal puncture was of great importance in diagnosis, as the Price-Jones curve showed a slight shift to the right, and microcytosis was not a feature (Graph 8). This, of course, may be due to the fact that the anæmia was not severe. It is often possible to establish the diagnosis from therapeutic tests. Provided really efficient iron preparations in adequate doses are used, successful treatment would indicate idiopathic hypochromic anæmia, just as successful liver therapy is pathognomonic for pernicious anæmia. The following case serves as an example:—

CASE 6. A 43-year-old agricultural worker felt weak, tired and giddy and could no longer do his work properly. Previously he had had only measles and influenza. During the last few years his diet consisted almost

entirely of bacon, ham and fat and relatively little vegetables, salad and fruit On admission to hospital the skin and mucous membranes were sallow and pale There was no evidence of blood loss. Chest and abdomen: normal. No achlorhydria Temperature; normal.

Blood: October 5th R.B.C. 3·39 millions, Hb. 56·1% = 9·0 g.%, C.I. 0·82, W.B.C. 4,460, eosinophils 5·5%, stab forms 1%, segmented polymorphs 65%, lymphocytes 23%, monocytes 5·5%. Platelets 208,000 Bleeding time 3 min, clotting time 3 min 15 sec. (Burker) Sedimentation rate (Westergren) 11–26 mm (1 and 2 hr.). Serum protein 7·72%, albumen/globulin = 65/35 Serum bilirubin direct negative, indirect 0·8 *mg.%*

Sternal Marrow Procrythroblasts 0·5, normoblasts 45·5 per 100 white cells; myeloblasts 1%, promyelocytes 2·5%, neutrophil semi-mature myelocytes 4%, mature myelocytes 15·5%, metamyelocytes 11%, stab forms 18·5%, segmented polymorphs 21% [illegible] myelo-

plasmoblasts 1%, proplasmocytes 0·5%, plasma cells 3·5%, reticulum cells 0·5% A picture of moderate hyperplasia of erythropoiesis.

With treatment by Ferrostabil and ordinary hospital diet rapid improvement took place. On November 10th R.B.C. were 5·4 millions, Hb. 95% = 15·2 g.%.

This, therefore, was a case of severe hypochromic anæmia with anisocytosis and microcytosis, which improved rapidly with iron treatment (Ferrostabil) Though there was no achlorhydria, the diagnosis of hypochromic, primary iron-deficiency anæmia was justified, because other causes had been excluded (*hæmorrhages, infections, tumours*) Possibly it could have been an anæmia of alimentary origin or from vitamin deficiency, but the success of iron treatment without extra vitamins in the diet would favour our diagnosis. The myelogram with moderately increased numbers of normoblasts (45·5%) is also in favour of idiopathic hypochromic anæmia

The ætiology of idiopathic hypochromic anæmia has been investigated by numerous workers Hirschfeld (1935), Thiele and Kuhl (1938) and Lundholm (1939) have noted familial tendencies to anæmia Schmidt (1931) succeeded in reproducing the family disposition to anæmia experimentally; in animals a prolonged iron-free diet did not cause anæmia, but the continuation of this deficient diet through several generations succeeded in doing so, and the severity of the anæmia increased from generation to generation. He attributed the anæmia to the lowered amounts of iron transmitted during intra-uterine life Later it became apparent that 80%–90% of all cases of idiopathic hypochromic anæmia occurred in women (Witts, 1931, Wintrobe and Beebe, 1933, Lundholm, 1939) Contrary to previously held beliefs, Schulten (1934), Britton (1936), Thiele and Kühl (1938) and Lundholm (1939) found that occasionally men also were affected According to Thiele (1938) men who have had rickets are especially predisposed to this hypochromic anæmia The very high proportion of

women in whom the hypochromic anæmia is first noted at the ch of life gave rise to a suspicion that there might be some relatio between the disease and the endocrine glands. Nolen (1925) more recently Feuchtinger (1941) suggested a hypofunction o genital glands, Meulengracht (1932) suggested thyroid deficienc a cause.

Moore *et al.* (1937), Heilmeyer and Plötner (1937) and Vannotti Delachaux (1942) measured the degree of iron deficiency by chen methods, and attributed great importance to it in the ætiolog idiopathic hypochromic anæmia. They found the iron conten serum to be $20\gamma\%$–$50\gamma\%$, instead of the normal average of $90\gamma\%$ women, and $125\gamma\%$ in men. Heilmeyer and Plötner, and Moore his colleagues have proved that the part of the serum iron whic *not bound up in hæmoglobin is decreased.* As this particular i fraction represents the source for the production of hæmoglo decrease of it may lead to a lowered production of hæmogloi Heilmeyer, Vannotti and others state that apart from the i deficiency in the blood, tissue hyposiderosis is an important facto the production of the disease. Total blood iron is about 2·5 g., tis iron 1–2 g. In the tissues, iron plays the part of a catalyst Lint (1929) was able to prove that a diet deficient in iron did not depl the body stores, because a healthy person can maintain his iron le even when its consumption is low, by decreasing its eliminati Albers (1942) states that true iron deficiency can occur only af hæmorrhage, and in other conditions only a relative iron deficien develops. Heilmeyer (1942) on the other hand, assumes that an ir deficient diet may lead to iron deficiency, when an increased dema for iron is present at the same time. Such increased demand in adu may be due to blood loss, infections and other diseases Heilmey attributes great importance to menstrual losses through the years a suggests the iron deficiency observed by him and Plötner and by Moo and colleagues may be due to repeated loss of blood According Micheli (1936) true iron deficiency can occur only in childhoo There are three ages in life, during which increased demand may le to manifestations of iron deficiency severe enough to produ disease :—

The age of rapid growth of sucklings and small infants

At the end of growth, at the onset of menstrual periods

At the menopause

These age groups correspond to the well-known forms of iro deficiency anæmia —

Anæmia of Sucklings and Small Infants (Glanzmann, 1937 Josephs, 1938), especially when fed on cow's or goat's milk It is often called alimentary anæmia or infantile chlorosis and occurs in town children between the ages of 4 months and 4 years (Mackay, 1935). This anæmia may be very *severe and depends on iron*

deficiency. If such severe cases, as those described by Glanzmann, are not suitably treated with iron or diet, death may ensue. Walthard (1936) proved the deficiency of iron in the organs by histo-chemical methods in one case. It is not yet ascertained whether, apart from iron deficiency, a deficiency in copper plays an important part, as presumed by Parsons (1933), Schiff *et al.* (1933), Hawksley (1936), Roth (1936) and others.

Chlorosis of Young Girls. Patek and Heath (1936), Heilmeyer and Plötner (1937) have demonstrated the iron deficiency quantitatively. According to Denecke and Kohlbrok (1936), Heilmeyer (1938), Alsted (1941) and several Russian authors it has once again become more common. Heilmeyer (1938), Waldenström (1941), and Thaddea (1943), believe it to be the juvenile form of iron deficiency anæmia. We have not seen any cases so far.

Menopausal Anæmia. Finally the anæmias of the third age

The Anæmia of Advanced Age may be mentioned here as a fourth group. It is usually due to a deficiency of iron in the diet and the inefficient use made of it. Barasciutti (1937) found, and we have confirmed, that iron usually cured this anæmia, especially when Vitamin B_1 and Vitamin C are also given. Sternal marrow in this fourth group also shows normoblastic hyperplasia.

Iron deficiency may be caused by inadequate intake Wintrobe and Beebe (1933) suggest this because of the greater prevalence of anæmia among the poorer, rather than the richer, sections of the population. More important is the poor absorption of iron, which may be attributed mainly to lack of acid or even total achlorhydria, according to Bethell *et al* (1934), Heilmeyer (1938), Vannotti and Delachaux (1942). Salvesen (1932) maintains that about every third woman with achlorhydria sooner or later becomes anæmic Other factors may be at work, as shown by the curious fact that though men and women are affected about equally by achlorhydria, the far larger proportion of anæmias occur in women Heilmeyer (1938) suggests the loss of blood during the 1943)

is a

ome

anæmic after gastrectomy in spite of achlorhydria, and also that iron is effective therapeutically even when given without hydrochloric acid and pepsin, though perhaps more slowly and less completely (Heilmeyer and Plötner, 1937, Moore and colleagues, 1937, Skouge, 1939, and ourselves). These last findings in particular emphasize the great importance of the normal gastric secretion, which has been pointed out recently by Karczag (1938) in a very careful study Wollheim (1944) considered that in addition

to Castle's factors, other factors are necessary for proper erythropoiesis. Mettier, Kellog and Rinehart (1933) show the importance of the normal gastric juice by the therapeutic effect of food predigested by normal gastric secretion in anæmic patients.

Observations on patients after gastrectomy have also demonstrated the great importance of the gastric secretion Rieder (1934) found that a third, Gutzeit (1932) and Dedichen (1934) that half, and sometimes more of the patients who had undergone resection of the stomach became victims of anæmia, usually of the hypochromic variety. Gordon-Taylor *et al.* (1929) found anæmia in 16 cases out of 52, Morley and Roberts (1928) in 12 of 42, but Henschen (1930) found only 3 of 77 cases According to Valeri (1909) and Seyderhelm (1929) the diminished iron absorption is due to a speed up of peristalsis and a quicker passage of the intestinal contents. Thiele (1938) and Heilmeyer (1938) observed this in idiopathic hypochromic anæmia, Gordon-Taylor *et al* (1929), Henschen (1930) and Scheidel (1930) proved it by X-rays after gastrectomy. Thiele (1938) and Vannotti and Delachaux (1942) believe that the elimination of the duodenum by causing a rapid passage of food might be responsible for the deficient absorption Hemmeler (1942) has observed satisfactory absorption of iron after gastrectomy when he attempted rapid saturation by intravenous injection. But both he and Morley and Roberts (1928) believe that the lack of acid is the cause of anæmia, owing to insufficient ionization of the iron in the diet. Fowler and Barer (1937) found that achlorhydric patients show a negative balance of 4 mg, after oral administration of 11 mg of iron daily, while patients with normal acid secretion after taking 10 mg show a positive gain of 1 mg Finally it may be mentioned that gastric ptosis, which is often observed in chlorosis, may lead to a poor absorption, but on the other hand the ptosis may be a consequence of chlorosis A vicious circle may be established, which may lead to inadequate utilization of the iron. One could almost call this anæmia "achrestic," as the iron offered to the organism cannot be utilized properly in spite of the presence of acid in the gastric juice According to Hochhaus and Quincke (1896), Schittenhelm (1940), and Vannotti and Delachaux (1942), iron altered by the gastic juice is absorbed in the duodenum, according to Hemmeler (1939) the jejunum also takes part in this process The important position of the gastric function is thus firmly based on numerous investigations and clinical observations

The diagnosis of iron deficiency anæmia is usually easily established by the blood picture and an estimation of the mean corpuscular hæmoglobin concentration by Wintrobe's method and the estimation of the serum iron It must not be forgotten that there are atypical cases as regards the blood picture, and that the iron level in the serum, as shown by Hemmeler (1944) may undergo appreciable diurnal variations These variations may be regulated by a neuro-

vegetative mechanism In 25 men on an average there were 44y% variations, and in women 31y%. The morning values are higher owing to an increase in the vagal tone. The iron level should not be estimated during bodily exertion, as at such times it is often lowered, as observed by Vannotti and Markwalder (1939) *This lowering* was shown by Delachaux and Ott (1943) to occur only at the beginning of exercise in fit people. In many cases sternal puncture is therefore particularly useful in differentiating idiopathic hypochromic anæmia from pernicious anæmia and pernicious-like states. Transitions from iron deficiency anæmia to pernicious anæmia, or rather to megalocytic anæmia, have been reported by Lenhartz (1925), Witts (1932), Jagić and Klima (1937), Meulengracht (1938), Schulten (1939), Waldenström (1940), Miller and Dameshek (1941) and Whitby and Britton (1946). This has been explained by Heilmeyer (1938) by the ultimately common basis of both diseases, namely disorder of the gastro-intestinal canal Leitner (1948) reported a case of idiopathic hypochromic anæmia undergoing transition to pernicious anæmia In the sternal marrow the tissue basophil cells were increased, and according to him this indicated a profound damage to the marrow He also believes that the ætiology is largely gastro-intestinal Achlorhydria results in the insufficient ionization of the iron in the food and leads to hypochromic anæmia, and later to complete achylia with absence of the Castle factor and thus causes the development of pernicious anæmia. It is possible that progressive insufficiency of the gastric secretion is much more common than thought hitherto One is tempted to use the words "pre-pernicious stage" of pernicious anæmia, with achlorhydria and hypochromic anæmia, when dealing with such cases.

[illegible]

and Zerfas (1934) Pregnancy involves an expenditure of 500 mg iron, as estimated by Fullerton (1936), which naturally produces an increased demand for iron. As in such cases the blood picture may not be characteristic, the diagnostic importance of sternal puncture is evident (Segerdahl, 1935)

Summary. (1) In the diagnosis of idiopathic hypochromic anæmia sternal puncture may occupy a decisive place Moderate hyperplasia of normoblasts of all ages, and eventually in severe cases, also of the proerythroblasts, is characteristic.

(2) In severe anæmias basophilic normoblasts predominate.

(3) In achlorhydria without anæmia basophilic, polychromatic and orthochromatic normoblasts are often found with a wide rim of cytoplasm which would indicate a definite relationship between gastric secretion and erythropoiesis

Anæmias of Vitamin Deficiency

The megalocytic anæmias due to deficiency of the extrinsic factor belong to a certain extent to this group, but have been discussed with the pernicious-like anæmias (p. 94) Vitamin deficiency is often accompanied by iron deficiency.

In Vitamin A deficiency, Kossler and Maurer (1927) noted anæmia, Euler and Malmberg (1939) noted a decrease of the number of reticulocytes in rats, and Anagnostu (1939) and Abbott and Ahmann (1938) observed marrow atrophy, or rather a jelly-like degenerative process. Blackfan and Wolbach (1933) report marrow atrophy in the human while Mainzer and Joel (1938) found many early and late normoblasts in the sternal marrow of 5 cases. Their cases were children with considerable anæmia (Hb. less than 50%), which began not unlike Lederer's anæmia with pyrexia and leucocytosis; they did not show evidence of hæmolysis, but rather a depression of hæmopoiesis, as indicated by low reticulocyte counts. In two children the anæmia was cured by an increase of Vitamin A in the diet, and in the others by Vitamin A and blood transfusions. Stodtmeister and Hock (1942) found that Vitamin A deficiency led to slight or moderate anæmia, which they believed to be due to increased hæmolysis owing to hyperactivity of the reticulo-endothelial system. They also found neutropenia (as did Abbot *et al.*, 1939), and thrombocytopenia.

Anæmia from Vitamin B_1 deficiency has already been discussed. Whipple (1928) working with animal experiments has concluded that Vitamin B_1 (thiamine) has a stimulating action on the production of hæmoglobin Though anæmias from thiamine deficiency are not known, Vannotti (1946) attaches great therapeutic importance to Vitamin B_2, especially in iron deficiency anæmias. Fouts, Helmer, Lepkowsky and Jukes (1938) observed microcytic anæmia in dogs with Vitamin B_6 deficiency

According to Mettier, Minot and Townsend (1930) and Israëls (1943), hypofunction of the marrow occurs in Vitamin C deficiency, while Harris (1928) found hæmorrhages, a development of fibrous tissue and a gelatinous degeneration. Euler and Malmberg (1939) noticed an increase in reticulocytes in guinea pigs after large doses of Vitamin C in the diet, and consider Vitamin C helped in the production of hæmoglobin Heilmeyer and Plötner (1937) and Rominger (1937) believe that Vitamin C is most important for iron-metabolism, by protecting the ferrous form from oxidation After the administration of Vitamin C, Seyderhelm and Grebe (1935) observed reticulocytosis, which they thought was due to stimulation of passage of mature forms into the blood stream *It is to be noted* that reticulocytes show granularity of the vital granulation in stimulated reticulocytosis Such cells are mature reticulocytes "Ball of wool" and "meshwork" *forms occur in regenerative reticu-*

locytosis. They are primitive forms of reticulocytes. Weber (1939) noted reticulocytosis in cases of scurvy after giving Vitamin C and considered this was a sign of regeneration. Babudieri and Perosa (1938) observed hyperplasia of the marrow reticulum in the bone marrow of guinea-pigs, which had had experimentally induced scurvy and had received Vitamin C subsequently. Mettier and Chew (1931) in experimental scurvy of guinea-pigs found marrow hyperplasia with an increase of normoblasts In man, Rohr (1940) found in anæmia from scurvy a rise in erythroblasts (54% late and 8% early normoblasts). Jennings and Glazebrook (1938), Schulten (1939), Henning and Keilback (1939) and Vilter *et al.* (1946) also report moderate increases of erythroblasts. McMillan and Inglis (1944) describe a normoblastic bone marrow in four cases and a megaloblastic reaction in one other.

In Vitamin D deficiency the position is not yet clearly understood. The anæmia of rachitic origin fails to respond to increased doses of Vitamin D alone, and therefore other factors, possibly some other deficiency occurring with the Vitamin D deficiency, must be incriminated (*e.g.*, deficiency of Vitamin C, or of iron) Seyderhelm (1929) has observed patients with considerable disturbance of Vitamin D and fat absorption who were apparently cured by the administration of Vitamin D. The beneficial effect of fish-liver oils in anæmias of children reported by Baar and Stransky (1928) may possibly be due to the Vitamin A also present in the oil. Seyderhelm and Tammann (1929) observed the development of anæmia in dogs with artificial biliary fistula and disturbance of absorption of Vitamin D. They responded to Vitamin D therapy

Vitamin K deficiency has been observed by Quick (1937) in chickens Anæmia subsequently developed and responded to the administration of Vitamin K In man, anæmia from Vitamin K deficiency has not been recorded

Summary Anæmia may be produced by deficiencies of certain vitamins. The bone marrow may be either hypoplastic, as in deficiency of Vitamin A, or hyperplastic, as in deficiency from Vitamin C or B_1

Other Iron-deficiency and Symptomatic Anæmias

In the chapter on idiopathic hypochromic anæmia we have discussed four types of anæmia, although strictly speaking achlorhydric anæmia in women approaching the menopause is the only one which should be included in that group A discussion of the four types, under a common heading, was justified hæmatologically, because in all of them iron deficiency developed either from inadequate supply or deficient absorption or both without any other recognizable cause In the anæmias of infants it is inadequate supply, in chlorosis and idiopathic hypochromic anæmia insufficient

absorption, in advanced age probably once more inadequate supply which is the chief cause. We deliberately omit increased demand as a factor, as supply must be adjusted to the demand. Hæmatological differentiation of the various forms is hardly possible. Both blood pictures and sternal punctures yield similar results. Individual clinical manifestations only justify the division into various types within the pathological picture of idiopathic hypochromic iron deficiency anæmia, as we would prefer to call this disease. The dead-white or greenish colour of the skin in chlorosis, the Plummer-Vinson syndrome in idiopathic hypochromic anæmia, cracks in the corner of the mouth, which are probably due to Vitamin B_2 deficiency, may contribute towards differentiation of *types rather more than slight differences in the blood picture.* Apart from these primary or idiopathic iron deficiency anæmias, there are also secondary iron deficiency anæmias, the cause of which, *e.g.*, hæmorrhage, infections, tumours, etc., may be discovered. Neither blood picture nor myelogram is characteristic in these anæmias. *Therefore they will be discussed with the underlying* diseases, and not as a separate group of their own

Apart from the iron deficiency anæmias, the following sections will deal with toxic, hypoplastic, aplastic and hæmolytic anæmias. They may often be differentiated from the primary and secondary iron deficiency anæmias by an estimation of the serum iron. According to Heilmeyer and Plötner (1937), Stodtmeister and Büchmann (1941) and Vannotti and Delachaux (1942), hæmolytic and aplastic anæmias usually have a normal or even raised serum iron level In the sternal marrow in symptomatic anæmias, to which group the secondary iron deficiency anæmias belong, Klima (1938) found an increase in immature forms (proerythroblasts) *indicative* of greater demands on the marrow. According to Introzzi (1935) the findings in the marrow are usually in keeping with the severity of the anæmia, and early cell forms occur in profound anæmia Tzanck and Dreyfuss (1937) classify all anæmias even including pernicious and hæmolytic anæmias by the proportions of nucleated red cells and white cells in the marrow. They distinguish three groups :—

(1) Where the ratio of red to white cells has been changed from 1 5 – 1 3 to 1 . 2 – 1 1

(2) Where it is lowered to 1 10

(3) Where the number of erythroblasts approaches 0

The first group is claimed to be hypochromic, the second comprises the megalocytic anæmias, and the third is normochromic From considerations of pernicious anæmia as discussed previously, it is obvious that such a scheme cannot be applied generally Stodtmeister (1937) has reported very similar reactions in the bone marrow in anæmias of diverse origin In post-hæmorrhagic anæmias, anæmias from infections, such as subacute bacterial

endocarditis and others, he found that any anæmia which responded to any therapy of any type showed a considerable increase of normoblasts in the sternal marrow at some period. As would be expected, this normoblastic crisis preceded the reticulocyte crisis in the blood and depended less on the number of erythrocytes than on the low hæmoglobin content. He also believes that erythroblastic hyperplasia is not always evidence of regeneration, but may be due to a disturbance of maturation. According to Fieschi's (1940) observations, as well as our own, this is the case only when some deficiency factor is at play. We have also examined supravital preparations of sternal marrow for changes in the number of reticulocytes and often found an increase In our experience a marked increase of reticulocytes in the marrow should be regarded in the same way as a normoblastic crisis. Ungricht (1938) carried out extensive observations in an attempt to solve the problem of the reticulocyte picture in the marrow He found that a reticulocyte increase in the marrow did not always indicate regenerative activity, in the presence of normal erythrocyte figures, it could even suggest damage of erythropoiesis, analogous to a shift to the left in the myeloid series.

Piechl (1941) divides the anæmias into two groups :—

(1) Anæmias of marrow origin (replacement of hæmopoietic marrow by space-filling lesions, toxic damage)

(2) Anæmias of extramedullary origin (hæmorrhage, hæmolysis)

In the first group the number of erythroblasts is low, in the second raised. In anæmias with good prognosis, he observed an increase in juvenile eosinophil leucocytes in the marrow, while the mature eosinophils remained at the same level as the eosinophils in the blood He therefore claims a relationship between the eosinophils and hæmopoiesis This classification, too, is not generally applicable In toxic marrow damage and in tumours, we have at times found an increase of erythroblasts in the marrow, though hypoplastic reactions were more frequent Habelmann (1941) presumes that in some anæmias a sort of obstruction to delivery occurs in the marrow, so that storage of erythroblasts of varying degree of maturity occurs at first without disturbance of maturation This might be due to marrow insufficiency owing to too rapid peripheral demand for erythropoiesis In hæmorrhagic anæmias this obstruction depends on the amount and the duration of the hæmorrhage, in anæmias from malignant disease it comes into play even when the peripheral blood picture shows little change from the normal, and is possibly due to the tumour metabolism He believes that liver and iron are completely worthless in these circumstances, and that the only hope for success lies in blood transfusions Habelmann's theory of this marrow obstruction, however, is not intelligible without a disturbance of maturation,

because nucleated red cells are not released into the blood stream except in certain circumstances. Though such schematic classifications are attractive, it is clear even from these short notes that they do not answer the demand for academic or practical accuracy.

Certain characteristics are common to all anæmias Iron deficiency occurs in the anæmias secondary to infection or intoxication Iron also is a general stimulant of hæmopoiesis, as stated by Heilmeyer and Plötner (1937) and Goetsch, Moore and Minnich (1946). *Still more important as a* marrow *stimulant* is oxygen deficiency, especially in the secondary anæmias. When the level of hæmoglobin falls, the consequent oxygen deficiency stimulates the bone marrow and results in a remission of the maturation arrest of the erythroblasts, which is so commonly seen in these anæmias, and therefore the anæmia becomes less severe. In tuberculosis and other infectious diseases, and in anæmia of nephritis, this critical level of hæmoglobin is 60% and anæmia in these cases rarely falls below it. The principal forms of anæmia do, however, differ according to the underlying disease.

ACUTE AND CHRONIC POST-HÆMORRHAGIC ANÆMIAS

Post-hæmorrhagic anæmias may be to a certain extent regarded as iron-deficiency anæmias, the cause of which happens to be known. In acute post-hæmorrhagic anæmia, clinical manifestations may not develop until the oligæmia which is the first threat to life has already been partially dealt with by compensatory measures of the organism, in particular by the passage of tissue fluid into the general blood stream Blood transfusions are the chief therapeutic aid to this physiological compensation, and the deficiency of iron need not be made good until later. In chronic post-hæmorrhagic anæmias, however, *the resulting state of iron deficiency is of primary* importance According to Brøchner-Mortenson (1943) the level of iron in the serum may fall to 10γ%. Piechl (1941) tried to discover how much blood need be lost before the bone marrow shows a reaction of increased activity. He found that when only 100 ml of blood were removed the number of eosinophils was increased and this increase was accompanied by an increase of normoblasts in the marrow. After the loss of 250 ml and over, the number of erythrocytes in the blood and the amount of hæmoglobin drops while the number of *normoblasts in the marrow definitely rises* He believes that the number of eosinophils in the marrow provides a very sensitive method of estimating stimulation of erythropoiesis We prefer to rely on the numbers of normoblasts Forssell (1939) has carried out most extensive studies into the changes in sternal marrow in post-hæmorrhagic anæmias He found figures for normoblasts higher in severe anæmia than in only moderate anæmia, but did not find really marked spontaneous regeneration during the first

two weeks after a hæmorrhage Dvorak (1928), Brugsch (1934), Tanzella (1937), Probst (1937), Heilmeyer (1938) and Bock (1940), found a tendency to macrocytosis in the peripheral blood starting on the third day after hæmorrhage Whitby and Britton (1946) consider that the lesser grades of this phenomenon are due to reticulocytosis, as reticulocytes have a larger diameter than mature red cells; the higher grades, which usually occur later, are caused by a vigorously reacting macronormoblastic marrow. In 20 patients with gastric and duodenal peptic ulcers, Bertola and Ravetta (1939) examined the sternal marrow and found figures of 33%–43% for normoblasts, and occasionally they noticed eosinophilia. Baldum (1939) reported a case of megalocytic anæmia from hæmorrhage from a duodenal ulcer, in whose marrow he found scanty "megaloblasts." Mallarmé (1937) and Henning and Keilhack (1939) found an increase in erythropoiesis, the intensity of which rose with the quantity of blood lost. Henning (1935) reported microerythroblasts in patients who lost blood from hæmorrhoids, but Thaddea (1943), in a paper on similar conditions, reports the presence of large early normoblasts. Whitby and Britton (1946) found a simple hyperplasia of the normoblastic series so that all forms of normoblasts became more numerous When the marrow reaction is especially vigorous, numerous large early normoblasts may be present. In 7 cases, de Weerdt (1939) observed a rise in normoblasts, and in the number of mitoses, but the maturation index remained unchanged

In chronic post-hæmorrhagic anæmias, Heilmeyer (1938) lays stress on the importance of iron deficiency In such cases he found a primitive marrow with basophilic normoblasts. Stodtmeister (1937) points out the rapid increase of normoblasts, amounting to a normoblastic crisis, when the anæmia is improving under therapy. According to Fieschi (1932) rather different circumstances exist in chronic loss of blood caused by ankylostoma duodenale. The marrow is usually hyperplastic with a disturbance of maturation and predominance of basophilic micronormoblasts; the number of immature forms is increased and mitoses, among which telophases predominate, are diminished The leucopoietic series shows only a slight shift to the left Fieschi believes that these changes are due not so much to the loss of blood, as to actual manifestations of deficiency of hæmopoietic factors According to Fieschi, the karyological curves show a slight lag, and Kienle (1943) found that the prophases were increased Renzi and Grasselini (1939) noted that in gastric and duodenal ulcer patients, the marrow was very cellular with sometimes normoblasts and sometimes the leucocytes predominating Plasma cells and eosinophils were invariably increased Linarzi and Schleicher (1940) noted an increase in the normoblastic series, the myeloid cells and also the megakaryocytes

We have observed various types of marrow reactions in hæmor-

rhage, and would like to illustrate them in the following 3 cases, which are characteristic :—

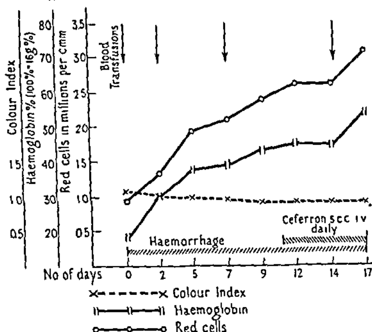
Case 7. R. A., aged 40, a labourer, had had trouble with his stomach since 1920; acid belching, burning pains in the abdomen, occurring 2–3 hours after meals. In 1926, a diagnosis of visceroptosis and hyperacidity was made. 1937: hæmatemesis. On January 4th, 1939, pains in the abdomen and acid indigestion became much worse. On the next day, tarry stools, vomiting coffee-ground material, pallor, noises in the ear, headaches, weakness. On examination: waxy pale man.

BLOOD. Hb. 19·4% = 3·1 g.%, R.B.C. 888,000 per cmm., C.I. 1·18, W.B.C. 7,820, juvenile forms 0·5%, stab forms 3%, segmented [illegible] nonocytes 6·5%; polychromasia, [illegible] Blood transfusion with blood from [illegible] group was started *immediately*. This had to be repeated three times as bleeding continued in spite of hæmostatic measures, Sippy diet, etc. Blood urea 30 mg.%; cholesterol 126 mg.%, calcium 8·3 mg.%, bilirubin direct and indirect, negative. [illegible] *ratio 95.5, sedi-* [illegible] noblasts 8·3, normo- [illegible] phagocytic reticulum cells 2·0%.

This picture shows a marked hyperplasia of erythropoiesis, in *fact a normoblastic crisis, which was followed by an increase of* reticulocytes in the peripheral blood to 10%. A certain amount of shift to the left in the myeloid series was also observed. *Another interesting fact* was the increase of eosinophil cells in the marrow, principally of the immature cells, while in the blood there was no eosinophilia but if anything a decrease of eosinophils. This indicates that primitive eosinophil cells are not released into the *circulation. The hæmorrhage did not stop for another fortnight,* after which iron therapy in the form of Ceferron intravenously was started, because administration by mouth still appeared contra-indicated, and a strict diet was maintained. Later oral iron was commenced, on the assumption that the iron reserves of the body might have been depleted by the considerable formation of normo-*blasts and by the deficiency in the diet resulting from the disease* process. Progress is shown in Graph 9.

Radiological examination after recovery from the critical stage of the disease showed an ulcer in the middle of the lesser curvature. Owing to the patient's dangerous condition, sternal puncture was not performed until after the third transfusion. It revealed a normoblastic crisis (Fig. 107) with marked normoblastic hyperplasia (134·4 per 100 whites). *Proerythroblasts were increased to 5 and* early basophilic normoblasts to 8. Compensation for the profound

anæmia could not be credited to the transfusions, which were solely a life-saving measure, but must be attributed to the marrow hyper-



GRAPH 9 Post hæmorrhagic anæmia in a case of duodenal ulcer with 19 4% = 1 1 g % hæmoglobin and 888,000 erythrocytes Improvement after repeated blood transfusions and iron therapy

plasia This was borne out by the subsequent reticulocyte crisis in the blood, due to the maturation of normoblasts into reticulocytes The newly formed red cells had a normal fragility, there was no excess of bilirubin in the serum. The erythrocyte sedimentation rate, in spite of the profound anæmia, was barely raised (15 mm.) owing to the hypoproteinæmia with reduction of globulin (serum protein 5 2 g %, albumen-globulin ratio 95 . 5). There was also hypocalcæmia (8 3 mg %) and hypocholesterolæmia (125 mg %) A normoblastic crisis usually occurs in severe anæmias, once the iron deficiency has been made good In other post-hæmorrhagic anæmias a very moderate normoblastic hyperplasia is sufficient to correct the anæmia This is shown in the following case —



FIG 107 Normoblastic crisis in post hæmorrhagic anæmia (Duodenal ulcer) (× 500)

Case 8. B A , a man of 33 years, had had abdominal pains and indigestion for years, with occasional vomiting On June 26th, 1938, he

had diarrhœa with tarry stools and vomited blackish material When seen at hospital there were no longer any tarry stools, but occult blood was present. Fractional test meal: free HCl: 27 ml. N/10 HCl per 100 ml. gastric juice, total acid 35 ml.

BLOOD. R B.C. 3·2 millions, Hb 71·2% = 11 5 g.%; W.B C. 4,300; basophils 3%, eosinophils 3%, stab forms 1%, segmented polymorphs 58·5%, lymphocytes 30%, monocytes 4·5%

STERNAL MARROW. Proerythroblasts 1·5, early normoblasts 2, normo-

reticulum cells 0·25%.

This is a case with moderate hyperplasia of the normoblasts and we interpreted this favourably for the prognosis. The anæmia improved rapidly, hæmorrhage soon stopped altogether and the *patient was discharged after 14 days. Thaddea (1943) has observed* normoblastic as well as macronormoblastic reactions in cases with hæmorrhage, but when loss of blood was comparatively slight he did *not find any appreciable hyperplasia of the normoblasts.* We have obtained similar results. On the other hand, the following case of chronic loss of blood showed a hypoplastic reaction, which we regarded as an unfavourable sign. Kienle (1943) holds the same views on this point

Case 9. B. M., a woman of 35 years, a painter of pottery, had been ill since 1934 with diarrhœal attacks, lassitude and loss of weight The frequent motions, up to thirteen times daily, later became slimy and mixed with blood, she developed abdominal pain, loss of appetite and a feeling of fulness. When examined she showed subnormal temperature, and ulcerative colitis was found by sigmoidoscopy Electrocardiogram normal

BLOOD. R.B.C. 3 9 millions, Hb 90% = 14·4 g %, C I 1·15, W B C 6 020. basophils 1%, eosinophils 1·5%, stab forms 3 5%, segmente

Is 0,
early 1%,
promy ytes
11·5%, metamyelocytes 16%, stab forms 14·25%, segmented polymorphs
eosinophil metamyelocytes 2 5%,
13·5%. monocytes 1 5%.

slight myeloid shift to

the disease was rapid, treatment made no appreciable difference It consisted of carbohydrate-free and fat-free diet rich in proteins *Larosan,* Redoxon, Benerva, injections of calcium gluconate, retention-enemata with Dermatol, Vogan and cod-liver oil, and blood transfusions Only five months later, after a diet of apples only was given on certain days, a régime suggested by Moro-Heisler, and Lacteol added, *did the improvement* become noticeable

2ND STERNAL PUNCTURE (October 10th, 1939) Showed evidence of improvement of erythropoiesis: early normoblasts 0.5, normoblasts 13 per 100 white cells; myeloblasts 1.5%, promyelocytes 2%, semimature myelocytes 3.5%, mature myelocytes 11%, otherwise similar to the first puncture. The blood picture showed improvement in December, 1939, with 4.4 millions R.B.C. and Hb. 88.5% = 14.2 g.-%. One year later the patient reported again. Her general condition was quite good, she had gained 13 lb. in weight, could do her housework, and the ulcerative colitis as well as the anæmia appeared cured. (R.B.C. 4.7 millions, Hb. 96% = 15.4 g.-%.)

We have quoted three typical cases:—

(1) A severe, acute loss of blood from a peptic ulcer with extreme anæmia (R.B.C. 880,000, Hb. 19.4%), in which, after transfusions and iron therapy there was a normoblastic crisis in the bone marrow with more than 140 normoblasts per 100 leucocytes.

(2) A rather protracted but less severe loss of blood from the stomach with moderate anæmia (R.B.C. 3.2 millions, Hb. 71.2%) in which there was a slight increase of normoblasts.

(3) Chronic loss of blood over 4 years, from ulcerative colitis, in which normoblasts were diminished, and in which there was a sluggish response to treatment.

These cases led to the conclusion that generally there is no *uniform* reaction in the marrow in post-hæmorrhagic anæmias. The marrow reaction varies with the degree and speed of loss of blood and with the ability of the marrow to react. Sternal puncture does, however, help in differential diagnosis. In cases (1) and (2), for instance, a hypoplastic anæmia could be excluded and in case (3) idiopathic hypochromic anæmia could be ruled out. The second marrow biopsy in the third case showed a slight, though definite increase of normoblasts, which was taken to mean a slight improvement in the regenerative faculty of erythropoiesis.

As regards marrow findings, a division into acute and chronic post-hæmorrhagic anæmias is quite justified, as an hypoplastic reaction, such as that in case (3), does not occur with acute loss of blood. Even in cases of severe hæmoptysis, which lasted for several days and ended fatally, we have always found that the normoblastic increase was the major one even though proerythroblasts and early normoblasts were also increased. In chronic loss of blood we have found a less pronounced increase of normoblasts, occasionally with the presence of basophilic normoblasts, which did not, however, exceed the number of polychromatic and orthochromatic normoblasts. Schulten (1934), Henning (1935), Thaddea (1943) and in single cases, Fieschi (1932), Heilmeyer (1938), Finger (1940) and other authors found hyperplastic reactions in chronic hæmorrhage. Hypoplastic reactions are much rarer and should be regarded as unfavourable prognostic signs; we believe they are an expression of marrow exhaustion. According to Kienle (1943) the occurrence of many immature proerythroblasts (delayed maturation curve) is an un-

favourable prognostic sign, while an increase of orthochromatic normoblasts (stimulated maturation curve), is a favourable one.

ANÆMIA IN INFECTIOUS DISEASES

While anæmia in infectious diseases is considered by several workers to be due to the toxic effect of the infection on the marrow, Heilmeyer and Plötner (1937) regard it as due to a state of iron-deficiency. Iron is stored in the reticulo-endothelial system in infections, and therefore the erythroblasts are not supplied with a sufficient amount of iron. Thus cell development and maturation become impaired or incomplete, and the erythrocytes are poorly hæmoglobinized Iron therapy, however, will not have its full effect until the infection is overcome. Reginster (1943) states that under these conditions iron therapy becomes redundant, as the amount of iron supplied in the diet is sufficient. In our experience we find that regeneration takes place much more quickly and safely when helped by parenteral administration of iron When the infection has cleared up iron is set free It increases in the plasma and is once more at the disposal of erythropoietic tissue Skouge (1939), Hemmeler (1939), Büchmann and Heyl (1939) and Schaefer (1940) have confirmed Heilmeyer's findings Saifi and Vaughan (1944) and Cartwright *et al* (1946) find that there is a disturbance of porphyrin metabolism and believe that the infection causes an upset of hæmoglobin synthesis. Thus Cartwright *et al*. showed that the plasma iron level drops within 48–96 hours of the development of the infection and before any anæmia appears Increases in the serum copper, the red cell protoporphyrin and urinary coproporphyrin excretion develop later. The plasma iron level could not be raised permanently by oral or parenteral iron but returned to normal only after the recovery from the infection and the disappearance of the anæmia Heilmeyer, Keiderling and Stuwe (1941) have also observed a rise in the plasma copper level at the same time as the iron level falls. This reciprocity is almost specific for infections and does not occur in such states as anæmia in malignant disease Sachs *et al* (1943) do not agree, however, with this statement The toxic effect of infection acting directly on the marrow certainly plays a very important part In some varieties of infection this toxic effect may be the dominant feature and thus apart from hypochromic anæmias of infection normochromic and even megalocytic anæmias may occur It is possible that in the latter class the infection may have some hæmolytic influence

In the sternal marrow of patients with malaria, Schretzenmayr (1938) has observed megalocytic anæmia with many proerythroblasts and megaloblasts, and in the blood hypersegmentation of neutrophils Schretzenmayr and Lancaster (1938) also noted

bone marrows containing proerythroblasts and megaloblasts in cases of chronic malaria. In eight further cases Kopasz (1942) noted punctate basophilia of normoblasts, which he thought was a sign of marrow irritation Bufano (1939) and Lebon and Manceaux (1939) in rather more acute forms of malaria reported a marked degree of normoblastic hyperplasia in the marrow and the presence of several megaloblasts and scanty myelocytes Benedetti and Merlo (1941) usually found marrow hyperplasia often coupled with anaplasia in cases of malaria with increase of erythroblasts in which proerythroblasts also took part. They also found megaloblasts and megalocytes, and have observed cases with marrow hypoplasia (myelo-asthenia), which were similar to Banti's Syndrome. The mesenchymal reaction in the marrow with hæmohistioblasts, histiocytes and plasma cells is of great importance in their opinion. In chronic malaria Bianchi (1940) found erythroblastic hyperplasia with many mitoses and preponderance of immature forms, megaloblasts and immature erythroblasts, which showed signs of histiocytic origin. When pregnancy complicated the picture of the disease, the bone marrow changes were much more pronounced He also found plasma cell hyperplasia Schretzenmayr reported that megalocytic anæmia in malaria responded well to liver therapy, which may be taken to indicate that these workers had really seen megaloblasts as we know them. Malaria probably induces a state of deficiency, possibly by damage to the liver or by upsetting the secretory mechanism of the stomach

Anæmia is fairly common in *acute septic infections* In our experience we have not seen hypoplasia or aplasia of erythropoiesis, not even in very severe infections, which eventually ended fatally Similar observations were reported by Galinowski (1938), Rohr (1940) and Thaddea (1943), the first in a case of *typhoid fever*, but Piechl (1943), in a case of *lung abscess* with severe anæmia, noted a reduction of erythroblasts Scaffidi and Molino (1941) report hypoplasia of erythropoiesis in *Malta fever* Sundberg and Spink (1947) describe the sternal puncture findings in 9 cases of active *undulant fever* There was some hyperplasia of normoblasts, myeloid cells, megakaryocytes, plasma cells and monocytes in most cases but the findings were variable Four of the cases showed typical granulomatous lesions Nanu-Muscel, Jonnesco and Valter (1931) and Tempka and Braun (1932) noted a megaloblastic-megalocytic anæmia in a *B coli* infection, but it is questionable if their definition of megaloblasts is the same as ours We have never found megaloblasts in septic infections nor has de Weerdt (1939) in his cases

The following case of subacute bacterial endocarditis may serve as an example for an acute anæmia in infection :—

Case 10. M B, a girl of 20, following a sore throat at the age of 7½ years, developed rheumatic polyarthritis and chorea, and a year later, iritis Cardiac incompetence was diagnosed at that time Since then the

patient has become breathless, even on slight exertion, and has had occa-sional recurrences of sore throat and arthritis. Three months ago sh again developed a sore throat with a temperature of 102·1° F., followe by subnormal t
pyrexia, and a
temperature wa
the whole precordium, but maximal at the mitral area. Electrocardio gram showed severe myocardial damage. The spleen was enlarged t one finger's-breadth below the costal margin and was soft. *Blood culture* and culture from sternal puncture were at first negative, but later grew *streptococcus viridans*.

BLOOD R.B.C. 2·72 millions, Hb. 58·2% = 9·4 g.%, C.I. 1·07; W.B.C 4,740; eosinophils 7%, stab forms 2%, segmented polymorphs 69%, lymphocytes 12%, monocytes 10%, toxic granulation was present. Sedimentation rate (Westergren) 76–114 mm. (1 and 2 hr).

[illegible]

blasts 29·5 per 100 white cells; myeloblasts 2·5%, promyelocytes 4·5%, semimature myelocytes 3·75%, mature myelocytes 14·25%, metamyelocytes 16·25%, stab forms 24·5%, segmented polymorphs 13·5%, eosinophil myelocytes 2·75% eosinophil metamyelocytes 2·25%, eosinophils 1·75%, basophils 0·5%, lymphocytes 4%, monocytes 0·75%, plasmoblasts 0·5%, proplasmocytes 1·25%, plasma cells 1·25%, endothelial cells 0·75%, lymphoid and phagocytic reticulum cells 2·5%.

In spite of the unhappy prognosis of the underlying disease and the severity of anæmia, the sternal marrow failed to show hypoplasia of erythropoiesis, though one would have expected a much higher percentage of normoblasts if the regenerative process of the marrow in an anæmia of this degree had not been impaired It is interesting to note the slight hyperplasia of the marrow reticulum cells The anæmia during the patient's stay in hospital improved to a level of Hb. 77·7% = 12·4 g.% and R.B.C 3·99 millions only after blood transfusion Prognosis remained most unfavourable as penicillin was not available, and shortly afterwards the patient died Prognostically, therefore, the findings in the marrow were of value only for the immediate future, whereas the underlying disease determined the overall prognosis.

Among chronic infectious diseases, *tuberculosis* is the only one of which we have been able to examine a sufficiently large number of anæmic patients Other authors have made similar investigations Labendziński (1938) has examined 40 cases and found that the number of erythroblasts in tuberculosis corresponded to the degree of anæmia Biernacki (1938) found normal figures for erythroblasts Pilgerstorfer and Seyfried (1938) descri cases with anæmia, and report hypoplasia of erythropoies they attribute to toxic marrow damage. imilar resul orted by Lanza (1938), Poli (1939), 940), Pie and T in (1944) We also fo e erythr often in numbers (see Tabl rin and , as

reduction in numbers, also observed a shift to the left among erythroblasts. Quattrin pointed out that karyorrhexis was rather frequent. Lanza (1930) in anæmic tuberculous patients found erythropoiesis to be slightly more active than in cases without anæmia. In toxic tuberculous processes he noted the presence of large erythroblasts; in cases with loss of blood, micro-erythroblasts were common. Bertola and Ravetta (1939) report an increase of orthochromatic and polychromatic normoblasts in anæmic tuberculous patients. László and Marton (1943) believe that very active erythropoiesis with proerythroblasts and early basophilic normoblasts is an unfavourable sign, but in our experience we failed to confirm this. The following case may serve as an example:—

CASE 11. G F., a man of 61 years, had suffered from pulmonary tuberculosis since 1933, but continued work until 1936. Had had cough, much sputum, lassitude, loss of appetite, shortness of breath. He developed abdominal pain, necessitating exploratory laparotomy. On examination he had bilateral active pulmonary tuberculosis with cavities and on the left side a much thickened pleura. Tubercle bacilli were present in the sputum

BLOOD R B C 3 46 millions, Hb. 76 7% = 12 3 g %, C.I. 1·02;

[illegible]

phagocytic reticulum cells 5%

We see here slight hypoplasia of erythropoiesis, without shift to the left This hypofunction is due to some toxic influence on the marrow. Stahel (1939) and Leitner (1940) have noticed an inhibition of the marrow function in splenomegaly Quattrin and Filla (1940) have differentiated the various forms of tuberculosis according to findings in the marrow, but we have been unable to confirm their findings in our own series of cases. They observed delay in red cell development in primary tuberculosis, marrow inhibition in exudative types of tuberculosis, and in productive types a sluggish development of red cells, with dissociation of production and development of the myeloid cells They found that there was considerable parallelism between lung and marrow findings Our cases of tuberculosis with anæmia are recorded in Table 6 In more than 100 cases of all forms of tuberculosis examined by sternal marrow biopsy we did not find any typical changes in the marrow picture which might be used in the differential diagnosis of tuberculosis whether early infiltration or cavities were present Normal or slightly lower figures for erythroblasts

TABLE 6

Erythropoiesis in Anæmic Tuberculous Patients

Case	Age in Years	Diagnosis	Blood: R B C in millions per cmm	Blood: Hb in % (100% = 16g %)	Sternal Marrow: Proerythroblasts	Sternal Marrow: Early Basophilic Normoblasts	Sternal Marrow: Intermediate and Late Normoblasts
					Per 100 White Cells		
1. A F.	24	Generalized caseous tuberculosis of lymphatic-hæmopoietic systems	3·27 2·82	80 60	0·6	0·6	11·3
2. S F	23	Late primary infection of right base.	3·0	74	0·3	1·3	21·6
3. E H	23	Bilateral pulmonary tuberculosis with pleurisy and effusion	3·93	83	0·3	0·6	15·0
4. D M	27	Miliary tuberculosis	4·1	84	0·6	1·3	16·6
5. L O	22	Abortive miliary tuberculosis	4·2	82	1·0	2·6	25·3
6. C W	21	Bilateral tuberculosis with cavities and effusions	4·0	85	0·3	0·3	11·3
7. S H.	20	Tuberculosis with cavity, right-sided pneumonia	4·5	68	2·0	2·5	35·5
8. Z B	30	Tuberculosis with cavities on both sides.	4·4	72	2·0	5·3	30·3
9. H M	16	Generalized caseous tuberculosis of lymphatic-hæmopoietic systems	2·36	38	0	0·3	6·3
10. R M	21	Cavities and infiltrations of left upper zone, pneumonia	3·45	68	0·6	2·3	35·0
11. S M	19	Miliary tuberculosis	4·7	83	0·3	1·6	14·6
12. H.B	21	Peritonitis, tuberculous salpingo oophoritis.	5·0	72	0·25	0·25	10·25
13. S E	30	Bilateral cavernous pulmonary tuberculosis	3·6	78	1·0	1·0	18·3
14. H K	25	Tuberculosis with bilateral cavities and effusions	4·32	82	1·3	1·0	16·3
15. G A	31	Tuberculous cervical adenitis	4·1	80	3·0	7·0	9·6
16. F V	26	Tuberculous peritonitis	4·8	75	2·0	4·0	34·3
17. N.J	44	Bilateral active tuberculosis with cavities	3·5	69	0·5	1·5	21·5
18. G F	60	Active pulmonary tuberculosis with lupus	3·46	76·7	0·3	0·3	18·6
19. G F	21	Active tuberculosis of left upper zone	3·8	68	1·0	3·0	49·5
20. C R	45	Active tuberculosis with cavity	3·21	61	0·6	2·0	18·0

predominate in Table 6 but in 5 cases we have seen increased numbers of erythroblasts. Very chronic cases usually show hypoplasia of the erythropoietic marrow portion. Comparatively frequently, even in patients with increased numbers of erythroblasts, we find the numbers of basophilic normoblasts increased at the expense of orthochromatic forms. This would indicate a maturation arrest, which in turn is recognizable in the blood picture by the

low reticulocyte counts. In the anæmias from infection, as in other varieties, oxygen deficiency is a very powerful bone marrow stimulant. This explains why in such cases anæmia rarely falls below Hb 60%.

This deficiency in hæmoglobin and the associated oxygen deficiency stimulate the bone marrow to increased activity, producing a slight remission of anæmia, or at least preventing further deterioration. During the active stage of infection iron is not a useful therapeutic agent, because it is caught in the reticulo-endothelial system, especially in the liver (Heilmeyer, Wintrobe, 1936) Iron will only become effective therapeutically after the active stage of the disease has subsided, but it must be remembered that the anæmia in these circumstances may remit even without iron.

We have recently observed a patient with tuberculous disease of the hip with cold abscess Her hæmoglobin for many months was 60%–63% and the red cells about 4 millions, irrespective of the administration of iron preparations. Once the abscess, which contained mixed bacteria, had been overcome by high doses of penicillin (1 million units in 5 days), the hæmoglobin rose to 76% in 3 weeks and later to 89%, without any iron therapy. In other cases ferrous iron was started when the infection was overcome We gained the impression that regeneration is aided by iron therapy.

From Table 6 it can be seen that there is often a reduction of erythroblasts in the marrow (of 20 patients, 12 had less than 20% normoblasts), while other cases may even show increased erythropoiesis Hypoplasia of erythropoiesis was particularly prominent in chronic cases Similar results have been found in anæmias due to other infections such as undulant fever, syphilis etc As a rule we found iron therapy unsuccessful during the highly active stages of tuberculosis We now give iron parenterally in the form of injections of Ferro-calcium (Sandoz) when the febrile phase has subsided with satisfactory results Reginster (1943) has given saturation doses of iron and found that in tuberculous patients, the more pronounced the hyposideræmia, the less the iron is absorbed. In febrile patients he observed particularly low serum iron levels

Meersseman, Friess and Lemaitre (1932), Collins (1935), and Veil (1939) have frequently found anæmia in *rheumatoid arthritis* Annoni (1938), Cattaneo and Cattaneo (1940), Rohr (1940) in *acute spondylitis* found normal or subnormal figures for normoblasts in the marrow Weitzmann (1941) found low figures in ankylosing spondylitis Kaether (1938) reports low reticulocyte counts in sternal marrow in *chronic arthritis and gout* (0 5% instead of 1·5%–2 0%) and attributes this to toxic damage. In our cases we have seen normal figures for reticulocytes and erythroblasts in sternal marrow, but so far none of our cases have presented any marked degree of anæmia.

Dell'Aqua (1942) reported 2 cases of severe anæmia in infections

with the Salmonella group of bacteria. In one case, which ended in complete recovery, he assumed accumulation of erythroblasts in the marrow without any release into the peripheral blood. The other patient died with a picture of aplastic anæmia.

We have already mentioned that in cases of *ankylostomiasis* Fieschi (1932) observed a maturational disturbance of the erythroblasts Schretzenmayr (1938) in ankylostomiasis and blackwater fever found hyperplastic normoblastic marrow, but in chronic cases of ankylostoma infestation he described a hypoplastic marrow. Tronchetti (1939) also found hyperplasia of erythropoiesis with disturbances of maturation in ankylostomiasis. In this type of anæmia from infections, iron-deficiency develops. The anæmia *responds well to the administration of iron*, even before the worms have been expelled.

ANÆMIA IN MALIGNANT DISEASE

In the anæmia of malignant disease, Heilmeyer (1942) postulates some mechanism similar to that of infectious disease; iron is stored in the reticulo-endothelial system, and this excess *storage does not leave sufficient for the production of erythrocytes* We believe that the anæmia in malignant disease is primarily due to toxic influence on the marrow In certain cases it must be presumed that other factors also play a part, such as occult loss of blood; and in the case of gastro-intestinal cancer, a deficiency of the intrinsic factor (Leitner, 1945), or a disturbance of absorption (Thaddea, 1943). Though anæmia in malignant disease often responds well to intensive doses of iron, as reported by Deleonardi and Re *(1940)* and *Heilmeyer (1942)*, it *is a typical secondary anæmia*, the outcome of which depends entirely on the underlying disease In our observations on 39 cases of carcinomata of the bronchus or stomach, we found hypoplasia of erythropoiesis most frequently, and it was especially well marked in the advanced stages of disease In the early stages the erythroblasts were sometimes increased A significant increase in the erythroblasts, however, was found in only 7 cases of our series of 39 The patients, whose skin is of a yellow-greyish colour, often look much paler than the blood picture would indicate, and their appearance suggests a toxic origin for the anæmia This characteristic colour of the skin is never found in any primary anæmia, and should at once suggest the diagnosis of malignancy.

Case 12. B F, 62 years, a stoker, became ill with cough temperature and pains in the left side of the chest towards the end of 1936 In the spring of 1937 pains increased, cough, expectoration, loss of appetite, vomiting, loss of weight, became worse *Since the summer of 1938 he* had shortness of breath, and a blood-stained sputum When examined he was pale Percussion note and air entry on the left side of the chest were diminished X-rays *showed* a dense shadow in the left lung field

and the heart and mediastinum were displaced to the left. Temperatures were subnormal

BLOOD. R.B C 3 3 millions, Hb 75 6% = 12·1 g.%, W B C. 20,500, eosinophils 1%, juvenile forms 2 5%, stab forms 5%, segmented polymorphs 87%, lymphocytes 4%, monocytes 5 5%. Sedimentation rate (Westergren) 121–136–144 mm. (1, 2 and 3 hr.)

1ST STERNAL PUNCTURE (June 24th) Early basophilic normoblasts 0 3, normoblasts 5 3 per 100 white cells; myeloblasts 1·3%, promyelocytes 2%, semimature myelocytes 9 3%, mature myelocytes 2%, juvenile forms 1
eosinophil myelocyte
megakaryocytes 1%,
3%, lymphoid reticu
endothelial cells 1%, fat cells 1·3% There was, therefore, definite hypoplasia of erythropoiesis

2ND STERNAL PUNCTURE (July 8th). Early normoblasts 1, normoblasts
2
1:
e
2

This myelogram is still more unfavourable than the previous one. The blood picture deteriorated correspondingly, anæmia increased to R B C. 3 04 millions, Hb 60 5% = 9 7 g.% Subsequent autopsy confirmed the diagnosis of carcinoma of the bronchus. There were no metastases in the sternum. In this case, therefore, we find an increasingly severe hypoplasia of erythropoiesis in the marrow, and a correspondingly progressive anæmia in the blood

In the marrow in anæmia from malignant disease Kienle (1943) observed a variety of reactions.

(1) Hyperplasia of granular cell series with shift to the left and suppression of normoblasts

(2) Hyperplasia of the marrow with primitive erythroblasts with a normal number of mitoses.

(3) Similar marrow (hyperplasia of erythropoiesis) with increased number of mitoses.

(4) Hyperplasia of normoblasts with mature forms (orthochromasia) usually when loss of blood occurred simultaneously.

(5) Hypoplastic marrow with evidence of degeneration of the granular cell series and maturation arrest of erythropoiesis

Kienle thinks that the first type is the commonest and in our own observations we have usually noted myeloid hyperplasia and shift to the left, while the red cells, as already stated, more often than not were hypoplastic We have not, however, found immaturity of erythroblasts In a case of leuco-erythroblastic reaction, which will be discussed later, hypoplasia of the marrow was present

Rohr (1940), Falzoy and Terzi (1940) and Fieschi (1940) in 2 cases of carcinoma of the stomach; Bertola and Ravetta (1939) in observations on 20 patients with carcinoma of the stomach, and Loeper *et al* (1939) in a case of carcinoma of the head of the pancreas with miliary metastases in lungs and liver, all found

The first two types are accompanied by iron deficiency and respond well to iron, but the third is refractory to iron therapy. De Langen (1942) in 3 cases found a low iron level (34·56γ%–78γ%) which improved promptly with iron. Giffin, Sanford and Szlapka (1918), Brown and Roth (1922), Löwinger (1938) and Nordenson (1938), however, do not regard anæmia of nephritis as a hæmolytic anæmia. Ceconi (1904) found the red cell resistance was even increased. In our own series we did not find increased fragility, but it is questionable whether examinations with hypotonic and hypertonic saline solutions cover all the factors concerned in hæmolysis In our cases the serum bilirubin was only rarely increased, but such an increase cannot be considered to indicate increased hæmolysis

Dacosta (1905) believed that the loss of albumen is of some importance, but Brown and Roth and Wilbur and Brown (1930) do not think this can cause anæmia. In nephritis, as will be shown in our own cases, the excretion of albumen is not great, and even in lipoid nephrosis with gross albuminuria, anæmia only rarely results In spite of this, the considerable loss of albumen in nephrosis may be a factor of some, if not of paramount importance. Grignani (1921) and Ashe (1929) incriminated the prolonged low protein diet as the cause of anæmia, but Griva and Asinelli (1932) found anæmia also in cases of nephritis which were discovered incidentally, and had not been on a special diet. On the other hand, Hoesslin (1890), Chittenden (1905) and Vinson (1922) failed to observe anæmia after a long period of low protein diet and Brown and Roth (1922) did not find any in fasting patients *Whipple and Hooper (1918) report a reduction in the rate of regeneration of erythrocytes in dogs on a high carbohydrate diet, Willi (1938) on a vegetarian diet, Leitner (1945) on a predominantly vegetarian* (Gerson) diet, and Marchal, Rouault and Deprez (1942) *on a diet poor in meat and fats. In accordance with these facts,* suggestions have recently been put forward by American authors (Fowler and Barer, 1937; Meyers *et al* 1938, and others) that a diet rich in meat should be given in order to combat idiopathic hypochromic anæmia They stress the importance of animal protein in hæmopoiesis Looper and Perreau (1939) believe that a variety of causes lead to the development of the anæmia, while Askanazy (1927) attributes the anæmia to the cachexia of nephritic patients

Some parallelism has been noted between the degree of anæmia and of azotæmia by Hamelin (1904), Parsons and Ekola-Strolberg (1933), Misske and Otto (1936) Alexeieff (1937), Townsend, Massie and Lyons (1937) Löwinger (1938), Nordenson (1938), Falzoi (1939) and Leitner (1941) Though the bone marrow is not an organ in which nitrogenous bodies are stored or deposited (Alexeieff), Parsons and Ekola-Strolberg found anæmia in every case of azotæmia, Misske and Otto in 90% of nephritic patients with, and in 44% of those without azotæmia.

and our own observations agree with these results. Marcolongo and Leone (1939) produced a fall of erythrocytes in thirteen of sixteen rabbits, and a fall of hæmoglobin in nine of sixteen animals with an ultra-filtrate of serum from uræmic patients, whereas ... produced only slight changes. The ... of xanthoprotein in the serum ... of aromatic products of intestinal putrefaction plays an imp... part. Ceconi (1904), Grignani (1921), Becher (1930), Naegeli (1931), Alexeieff (1937) and Löwinger (1938), hold the view shared by us, that the causal factor is the toxic action of the disease on the bone marrow and that azotæmia itself also plays a big part.

Before 1941, studies of bone marrow on anæmia of nephritis were relatively few in the literature. Since then several reports have been published, which have confirmed our findings, and the ... of the marrow in anæmia of nephritis is now more ... very active marrow ... also found reduced figures for erythroblasts ... ined a larger series of patients by sternal puncture and found reduction of erythroblasts in 11 of 17 cases, and an increase in the other 6. Nordenson (1938) in a series of 18 cases found subnormal figures for erythroblasts in the great majority. Similar results are reported by Michelazzi ... and Loeper and Perreau (1939) ... portion of red to white ... there was an increase of myelocytes at the expense of promyelocytes and metamyelocytes. Gingold, Comsa and Roman-Crivat (1938) in a case of severe azotæmic anæmia observed large numbers of normoblasts in the blood, while the marrow showed no sign of regeneration. The authors express the view that the marrow may ... of retaining immature cells. Nolli (1939) ... that the more severe ... picture of aplastic anæmia. Even in cases where ... increased figures of normoblasts, improvement of the anæmia was not evident, which phenomenon was attributed to a disturbance of maturation. Loeper and Perreau's (1939) findings of increased reticulocyte counts in the blood, however, do not tally with these observations and deductions. Giacchero and Belletti (1941) report inhibition of maturation in 12 patients, whose marrow was either normal or hyperplastic and tended to show raised normoblastic figures. Freschi (1940) has examined 2 cases, one of which showed a hæmolytic anæmia with a cellular marrow and the other marrow hyperplasia with karyological hypofunction, but without important changes of the maturation curve. He maintains that the cause of

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Some parallelism has been noted between the degree of anæmia and of azotæmia by Hamelin (1904), Parsons and Ekola-Strolberg (1933), Misske and Otto (1936), Alexeieff (1937), Townsend, Massie and Lyons (1937), Löwinger (1938), Nordenson (1938), Falzoi (1939) and Leitner (1941) Though the bone marrow is not an organ in which nitrogenous bodies are stored or deposited (Alexeieff), Parsons and Ekola-Strolberg found anæmia in every case of azotæmia, Misske and Otto in 90% of nephritic patients with, and in 44% of those without azotæmia,

and our own observations agree with these results Marcolongo and Leone (1939) produced a fall of erythrocytes in thirteen of sixteen rabbits, and a fall of hæmoglobin in nine of sixteen animals with an ultra-filtrate of serum from uræmic patients, whereas serum from healthy subjects produced only slight changes The degree of anæmia appeared to depend particularly on the amount of xanthoprotein in the serum. Becher (1930) believes the retention of aromatic products of intestinal putrefaction plays an important part Ceconi (1904), Grignani (1921), Becher (1930), Naegeli (1931), Alexeieff (1937) and Löwinger (1938), hold the view shared by us, that the causal factor is the toxic action of the disease on the bone marrow and that azotæmia itself also plays a big part.

Before 1941, studies of bone marrow on anæmia of nephritis were relatively few in the literature. Since then several reports have been published, which have confirmed our findings, and the function of the marrow in anæmia of nephritis is now more thoroughly understood. Scarlett (1929) found a very active marrow in some cases, but like Aubertin and Yacoel (1924) also found reduced figures for erythroblasts Löwinger (1938) examined a larger series of patients by sternal puncture and found reduction of erythroblasts in 11 of 17 cases, and an increase in the other 6 Nordenson (1938) in a series of 18 cases found subnormal figures for erythroblasts in the great majority. Similar results are reported by Michelazzi and Renzi (1937) in 17 cases , and Loeper and Perreau (1939) and Falzoj (1939) found a reduction of hæmohistioblasts and hæmocytoblasts as well as of proerythroblasts in the marrow. The proportion of red to white cells was altered in favour of the latter and there was an increase of myelocytes at the expense of promyelocytes and metamyelocytes Gingold, Comsa and Roman-Crivat (1938) in a case of severe azotæmic anæmia observed large numbers of normoblasts in the blood, while the marrow showed no sign of regeneration The authors express the view that the marrow may have lost the faculty of retaining immature cells Nolli (1939) examined 18 cases of acute and chronic nephritis and found that the more severe the kidney lesion, the nearer the marrow came to a picture of aplastic anæmia Even in cases where the marrow showed increased figures of normoblasts, improvement of the anæmia was not evident, which phenomenon was attributed to a disturbance of maturation Loeper and Perreau's (1939) findings of increased reticulocyte counts in the blood, however, do not tally with these observations and deductions Giacchero and Belletti (1941) report inhibition of maturation in 12 patients, whose marrow was either normal or hyperplastic and tended to show raised normoblastic figures Fieschi (1940) has examined 2 cases, one of which showed a hæmolytic anæmia with a cellular marrow and the other marrow hyperplasia with karyological hypofunction, but without important changes of the maturation curve He maintains that the cause of

TABL

Anæmi

Patient Age in years Complaint	1 W M 23 Acute nephritis			2 B R 30 Chronic nephritis		3 W H. 15 Subacute nephritis
Date	(1) 10 8	(2)	(3) 8 10	(1) 13 9	(2) 1 11	
Blood .						
R B C in millions per cmm.	3·2	2·6	4·0	2·8	2·2	3·8
Hæmoglobin % (100% = 16 g. %)	72	69·1	80	60·5	60·5	86·4
Colour index	0·93	1·3	1·0	1·08	1·37	1·1
Leucocytes per cmm	7,640		6,700	6,400		7,500
Basophils %	1·5		0·5			0·5
Eosinophils %	0·5		1·5	1·0		5·0
Stab forms %	2·0		0·5			3·0
Segmented polymorphs %	70·0		66·0	83·0		62·0
Lymphocytes %	20·0		25·5	14·5		24·5
Monocytes %	6·0		6·0	1·5		6·0
Plasma cells %						
Serum :						
Non-prot.-nitrogen mg %	65·0		9·0	80·0		38·0
Blood urea mg %	103·0			72·0		27·0
Alkali reserve vol %			45·0	48·0		
Bilirubin mg %	neg		neg	neg		neg
Sedimentation rate mm per hours 1 and 2	30/60		10/32	61/102		47/83
Sternal Marrow						
Proerythroblasts } per 100						2·0
Early normoblasts } white	1·5		2·0			8·0
Normoblasts } cells	3·0		23·5	18·0	7·0	34·0
Myeloblasts %	3·0		0·5	2·5	0·75	1·0
Promyelocytes %	2·0		1·5	1·0	3·25	4·5
Neutrophil semimature myelocytes %	3·0		1·5	0·5	1·75	5·5
Neutrophil mature myelocytes %	11·0		14·0	9·25	5·75	8·5
Neutrophil metamyelocytes %	17·5		11·0	13·25	11·0	23·5
Neutrophil stab forms %	22·5		25·0	29·25	14·5	15·5
Neutrophil segmented polymorphs %	17·5		26·5	23·25	34·0	17·0
Basophils %						
Eosinophil myelocytes %	2·0		2·5	2·0	4·0	4·5
Eosinophils %	0·5		1·0	1·0	2·25	6·5
Lymphocytes %	6·0		5·5	13·5	14·75	9·5
Monocytes %	1·5		1·0	0·5	1·5	1·0
Megakaryocytes %	0·5		1·0	0·25	0·25	1·0
Plasma cells %	1·0		2·0	0·5	2·25	1·0
Primitive reticulum cells %	0·5		0·5	1·25	1·75	
Phagocytic reticulum cells %			0·5	1·0	1·0	
Endothelial cells %					1·0	2·0
Clinical data						
Blood pressure in mm Hg	180/90		100/80	225/150	I.S.Q.	
Urine	Alb +		No alb	Alb 0·3%	I.S.Q.	
Sediment	Granular casts		Normal	Casts & R.B.C.s	I.S.Q.	
End result			Cured	*Deteriorated*		*Improved*

Nephritis

4 P. F. 43 Chronic nephritis (1) 16.9	4 P. F. (2) 15.12	5 A. C. 48 Uræmia	6 S. M. 23 Acute nephritis	7 S. D. 43 Uræmia	8 W. F. 32 Chronic nephritis (1) 16.9	8 W. F. (2) 12.10	9 M. W. 31 Acute nephritis	10 L. F. 44 Uræmia	11. J. G. Acute nephritis
4.1 85.3 1.03 5,900 0.5 1.5 2.0 77.5 15.5 3.0	3.9 78.8 1.0	3.0 81 1.35 8,600 2.0 6.0 2.0 68.5 15.0 6.5	3.9 80 1.02 9,760 1.5 76.0 15.0 7.5	4.2 86.4 1.03 8,800 1.5 13.0 11.5 52.0 17.0 5.0	3.0 86.3 1.1 7,680 1.5 2.5 77.0 16.0 3.0	3.3 70.2 1.1	3.8 88.5 1.1 9,400 0.5 0.5 66.0 24.0 9.0	5.9 121 1.01 10,940 0.5 1.5 3.0 71.0 14.0 10.0	4.1 80.4 1.0 8,250 0.5 1.0 3.5 68.0 23.0 4.0 0.5
63.0 68.0 43.0 neg 72,103		82–180 130.0 42–28 neg 24.56	50.0 32.0 48.0 neg 52,78	82.0 63.2 29.0 indir 1.7 72,103	150.0 130.0 41.0 neg 94 133	98.0 74.0 neg	36.0 23.5 neg 5.10	36.0 21.0 40.0 0.5 5.10	64.0 50.0 33.0 neg 34,69
2.0 1.0 0.5 3.5	0.5 0.75 27.75 0.5 0.5 2.5	2.0 16.0 1.0 5.5 10.0	0.5 1.0 16.0 1.5 3.25 4.5	0.5 0.5 7.0 2.0 1.0 2.5	14.0 2.0 1.5 4.5	0.75 4.0 33.0 0.75 3.75 5.0	1.0 1.0 17.5 1.25 1.55 3.75	1.0 0.3 15.6 1.3 1.6 1.3	0.5 2.0 16.25 1.0 2.25 4.25
10.5 19.5 15.5 30.5	11.0 12.25 17.0 35.0	17.0 40.0	1.0 17.0 15.5 26.0	4.0 2.0 12.5 28.0	9.0 10.0 23.0 22.25	8.75 18.75 14.75 19.5	20.75 14.0 21.0 19.5	13.3 5.6 13.3 20.6	12.5 16.0 20.5 21.5
2.0 2.5 8.0 2.0 0.5 3.5 0.5	0.5 3.25 2.75 5.75 1.0 0.5 2.75 0.5 3.0 0.5	5.0 1.5 12.5 2.0 0.5 1.0 1.0 0.5 0.5	3.5 1.75 9.5 0.25 0.25 5.5 0.5 0.25 1.0	0.5 2.0 1.0 17.5 3.5 0.25 1.0 1.5 1.5 3.0	2.25 6.5 9.25 0.5 0.25 3.75 1.5 1.5 0.5	5.25 1.75 9.75 1.75 0.25 3.0 1.0 1.0 1.0	2.0 2.0 8.0 0.25 0.5 4.0 0.5 0.25	0.3 3.0 2.3 16.6 2.3 1.3 4.6 2.6 2.6 1.6	0.5 2.25 2.0 7.25 1.75 2.25 4.5 1.25 0.5 0.25
260/130 Alb ++ Granular casts	160/100 Alb + Normal	260/130 Alb ++ Granular casts	145/70 Alb + Granular casts	170/100 Alb ++ Granular casts	165/120 Alb ++ Granular casts	Alb + Normal	150/95 Alb 0.6% R B C.s	200/140 Alb 0.3% Granular casts	180/95 Alb 0.2% Granular casts
Improved		Died	Cured	Very slow impr	Slight improvement		Cured	Died	Cured

the anæmia is to be sought at the stage of the stem cell, just as in aplastic anæmia, because the numbers of the polychromatic normoblasts were within normal limits, an observation which we have confirmed In all his 3 cases de Weerdt (1939) found hypoplasia of erythropoiesis, and Faarup and Soeberg-Ohlsen (1941) reported similar findings in chronic nephritis and nephrosclerosis. Young and Osgood (1935) in one case found hypoplasia of 9% normoblasts, but also reported 2 4% megaloblasts, this latter finding presumably being due to a nomenclature differing from ours. In 90 patients with acute nephritis, Büchmann and Stodtmeister (1943) found anæmia in 42, but only 6 had a hæmoglobin of less than 60% = 9·6 g.% They carried out a few sternal punctures and in chronic anæmia found impairment of erythropoiesis and a slight increase of leucopoiesis, corroborating our earlier results. In chronic uræmia there is a rise in serum iron, which they believe indicates a toxic-aplastic type of anæmia.

We have examined a number of patients with azotæmia by blood pictures and sternal punctures and correlated these findings with the degree of retention, as judged by non-protein nitrogen, blood urea, alkali reserve, indican and xanthoprotein. The results are shown in Table 7.

With the exception of Case 3, in which sternal puncture was not performed until evidence of nitrogen retention had regressed, lowered figures for normoblasts and absence of primitive forms, such as early normoblasts and proerythroblasts, were invariably found The number of mitoses was decreased, and the karyological curves showed varying degrees of inhibition with preponderance of the final phases of cell division These changes are more apparent in chronic nephritis, but become quite obvious in acute nephritis after a few weeks. In Cases 1, 4 and 9, which showed hypoplasia of erythropoiesis as shown by sternal puncture, the anæmia increased, but improved later as the underlying disease improved and the azotæmia regressed In Case 2 also, parallel with deterioration of the clinical state, anæmia became more pronounced; the marrow findings at the same time became less favourable and normoblasts fell from 18 to 7 per 100 white cells. In a patient with Boeck's sarcoid, erythropoiesis was normal (proerythroblasts 1, early normoblasts 2 5, normoblasts 25 25 per 100 white cells) The kidneys were affected, and there were casts and albumen in the urine The blood pressure was not raised, and there was no element of nephrosis, and no evidence of nitrogen retention The high red cell count in Case 10 was probably due to hæmoconcentration, or to the severe dyspnœa present or both

In the search for the causes of the anæmia and of the marrow changes, hæmolysis can be excluded on these findings. The fragility of the red cells in various concentrations of saline also revealed no abnormality, and the absence (excepting Case 7) of bilirubinæmia

tends to exclude increased blood destruction Anæmia was isochromic or normochromic, being hypochromic only in Case 1. Iron deficiency as the causal factor appears to be improbable, and the lack of therapeutic response to ferrous preparations favours its exclusion. Our observations failed to suggest low-protein diet as the cause of anæmia, but we have not treated cases of nephritis with a diet rich in proteins and so have no controls. Observations (Leitner, 1931) showed a slight fall of hæmoglobin and of the number of erythrocytes in cases treated by the diet suggested by Gerson and Sauerbruch Anæmia showed a definite relation to azotæmia, and to nitrogen retention. Whenever we found hypoplasia of erythroblasts in the marrow, the non-protein-nitrogen and blood urea were raised, and so were the serum indican and xanthoprotein (in Case 5 up to 63 colorimetric units) In Case 9 azotæmia had already passed the peak when sternal puncture was performed, but the erythroblasts were still slightly diminished in numbers Endogenous intoxication of the marrow by the retained products of metabolism most probably plays an important, though possibly not exclusive, part in the causation of anæmia

Summary. Sternal marrow in anæmia of nephritis shows hypoplasia, but rarely aplasia of erythropoiesis Leucopoiesis, apart from a slight metamyelocytic-myelocytic shift to the left, is unaffected, as also is megakaryocytopoiesis Certain cases showed slight eosinophilia and increase of plasma cells, which might indicate an allergic process. When the kidney lesion and the nitrogen retention improved, the anæmia also improved We are, therefore, dealing with an anæmia which is temporarily hypoplastic but not aplastic In progressive chronic nephritis the marrow showed an increasingly aplastic normoblastic picture

The anæmia does not respond to therapeutic measures, such as iron, arsenic or liver, until the underlying disease has begun to abate Sternal puncture allows us to gauge the tendency to regeneration or aplasia.

EXOGENOUS TOXIC ANÆMIAS

Apart from compounds originating within the body, there are numerous materials which may have a damaging influence on the marrow, some of them acting exclusively on it We confine ourselves here to the intoxications which are of importance in industrial medicine and to certain therapeutic remedies The knowledge of these materials is of importance, because hæmatological control in certain branches of industry and the administration of medicaments makes it possible to exercise efficient prophylaxis against marrow damage. The use of gold preparations, sulphanilamide and similar drugs, acetanilide, nirvanol, arsenic (salvarsan and other arsenicals), and industrial intoxications with lead, benzol, petrol, trichlorethy-

lene, carbon tetrachloride, arsenic, toluylenediamine and saponin may lead to aplastic or hæmolytic types of anæmia. These problems have been investigated by Gunther (1935), Gänsslen (1936), Matthes (1937), Sack (1940), Bomford and Rhoads (1941), Pein (1941), Schwarz and Teleky (1941), Heilmeyer (1942), Levin and Keddie (1942) and other authors.

Anæmia of Lead Poisoning

It is well known that intoxication with lead may cause anæmia. Anæmia from plumbism is not often a severe one, hæmoglobin values are usually between 40% and 80%. The colour index most often is less than unity, rarely more. Hypochromic anæmias have been described by Pellegrini (1935), and Pfeil (1940). According to Schmidt-Kehl (1927) anæmia in plumbism depends on increased destruction of erythrocytes, whose fragility is increased. Kin (1937) in animal experiments and Brookfield (1928) in man, found that with increasing dosage the damage from lead poisoning increased. Hamelin (1904), Grawitz (1911), Behrens (1925), Lewin (1929), Borchardt (1930), Lane (1931), Schilling (1933) and others found that punctate basophilia of the erythrocytes is characteristic for plumbism, but Teleky *et al.* (1919), Koelsch (1927), Naegeli (1931), Leitner (1941) and Heilmeyer (1942), report that it is neither a specific nor a constant sign; Hulst (1937) has observed it also in patients treated with gold and Myhre (1938) in cases treated with sulphanilamide. In our experience it may be present in the anæmias in old age and in malignant disease According to Meyer (1931) and Lehmann (1933), stippling may be absent on one day and present on another, and this has been well demonstrated by Whitby and Britton (1933) in experimental poisoning of rabbits Aub *et al.* (1925) observed variations in the number of stippled cells within the course of one day The exact meaning of punctate basophilia is still a matter of discussion, but the nuclear origin of the stippling has been suggested in animal experiments by Schmidt (1937), who found it only in nucleated red cells. We believe it is a manifestation of pathological regeneration

In bone marrow, in erythrocytes and in the excretions of patients with plumbism, Vannotti (1940) found an increase of porphyrin III, which he interpreted as a sign of pathological synthesis of hæmoglobin. Vannotti and Siegrist (1940) believe that lead inhibits the incorporation of iron in the hæmatoporphyrin ring, and the production of porphyrin is thus increased It follows, that erythrocytes, which contain hæmatoporphyrin and are produced by way of a disturbed regeneration are more fragile, and, therefore, are more easily hæmolysed We have compared punctate basophilia with the toxic granulation of leucocytes The

former is related to normal vital reticulation precisely as the latter is to normal granulation of leucocytes. Winkler (1937) claims that after small doses of potassium iodide the number of stippled cells increases, but Böhm and Fellinger (1936) failed to confirm this. Tischendorf (1939) believes, and we agree, that punctate basophilia is evidence of pathological regeneration. His opinion is that the nucleus is destroyed by karyorrhexis. Heilmeyer (1942) and also Thaddea (1943), however, believe stippling is identical with vital granulation and with polychromasia, and has only assumed a different visual picture owing to fixation with stains and stain dilution (Humperdinck, 1940). Whitby and Britton (1933) poisoned rabbits with phenylhydrazine and lead; the former produces marked polychromasia, the latter punctate basophilia. It was found in the same rabbit that whichever cell, polychromatic or stippled, predominated in the Leishman stained film, the sum of the two together was always parallel to the number of reticulocytes found in the supravitally stained film; that cells, such as normoblasts, which could be identified as the same in both Leishman and supravitally stained films showed polychromasia or stippling in the former and reticulum in the latter; that, by means of different stains, whatever the proportions of stippled and polychromatic cells might be (and this was largely a function of the stain), yet their sum total was always the same It was therefore deduced that stippling and polychromasia are both manifestations of reticulation and that the stipples are reticulum slightly altered by lead or other poison Rosegger (1936) has also shown that the isoelectric point is the same for stippling, polychromasia and reticulum, which indicates the identical nature of the three substances.

Apart from punctate basophilia the other hæmatological characteristics found in plumbism are anisocytosis, poikilocytosis and macrocytosis of the red cells, and a shift to the right of the Price-Jones curve Whitby and Britton (1946), however, found the mean corpuscular volume low or normal in most cases The leucocytes show some changes too Baader (1928), Muller (1938) and we ourselves have found lymphocytosis as an early sign, indicating damage of granulocytopoiesis.

Betti (1940) examined the changes in the bone marrow in rabbits injected subcutaneously with 1% lead-acetate. He found a myelocytic shift to the left of the leucopoietic series and a diminution of erythroblasts in the femoral marrow, and concluded that this indicated exhaustion due to the hæmolytic effects of lead Klima and Seyfried (1937), working on rabbits and guinea-pigs, observed erythroblastic hyperplasia in the marrow with early normoblasts and proerythroblasts in the early stages and marrow atrophy later.

In human sternal marrow, Alder (1939) and Markoff (1939) observed early normoblasts and erythrocytes with basophilic stippling, Klima and Seyfried (1937) noted hyperplasia of proerythroblasts

and normoblasts and Henning and Keilhack (1940) that basophil stippled red cells are more numerous in sternal marrow than in peripheral blood in the proportion of one stippled cell to nine normal ones. Leitner (1941) and Thaddea (1943) have been able to confirm this observation. Bentsath and Varga (1940) in a case diagnosed as nephritis, found 1·8% stippled red cells in sternal marrow, while no stippled cells were seen in the blood. Redondo (1941) reported *severe macrocytic anæmia and considerable erythroblastic hyperplasia* with normal maturation in the sternal marrow in a series of 8 patients. He also noted the presence of punctate basophilia. De Weerdt (1939) in 2 cases of chronic lead poisoning observed an increase in normoblasts, increased karyorrhexis, pyknotic nuclei and punctate basophilia. In our own cases we found increased karyorrhexis of the erythroblasts. Kienle (1943) reported increased *erythropoiesis with pathological cells and an increased number of* mitoses, but in our own material we were not convinced that karyokinesis was definitely increased. Pellegrini's (1935) observations about megaloblasts in the marrow in a "pernicious like" case of anæmia of lead poisoning has not been confirmed. Heilmeyer (1942), who also found erythroblastic hyperplasia, only reported erythroblasts similar to megaloblasts, but he emphasized the absence of typical megaloblasts. Perhaps Pellegrini's case was one complicated by pernicious anæmia, but toxic pernicious anæmia from exhaustion of the hæmopoietic principle seems most unlikely. We have never observed megaloblasts in the marrow, but only hyperplasia of normoblasts, as in the following case:—

Case 14. A 58-year-old lead worker. When examined, his gums showed a definite blue line, and his skin a peculiar greyish colour, and he had spastic constipation. The stools contained lead. There was hæmatoporphyrinuria. Though the patient looked pale, there was no evidence of anæmia. The pallor may have been due to a contraction of the vessels, which has been pointed out by Lewin (1929) in connection with the pallor in plumbism.

Blood. R.B.C. 5·6 millions, *Hb.* 102·6% = 16·4 g %, C.I. 1·0. W [illegible] 56% [illegible] (W [illegible] bla [illegible] promyelocytes 3%, semimature myelocytes 4·5%, mature myelocytes 7%, metamyelocytes 15·5%, stab forms 22·5%, segmented polymorphs 23·5%, eosinophil myelocytes 2·5%, eosinophil metamyelocytes 1·5%, eosinophils 4·5%, lymphocytes 12·5%, monocytes 0·5%, megakaryocytes 0·5%, endothelial cells 0·5%, plasma cells 0·5%.

Our findings of hyperplasia of erythropoiesis and of early normoblasts agree with those of *Klima and Seyfried* (1937), *Alder* (1939), Rohr (1940), Redondo (1941) Kienle (1943) and Thaddea (1943). We failed to find punctate basophilia in this case, but in two further cases with moderate anæmia of lead poisoning there were more stippled

red cells in the marrow than in the blood. Lymphocytosis in the blood picture was well marked in all 3 cases, but damage of the granulocytes was not apparent in sternal marrow.

Summary. The anæmia of lead poisoning belongs to the toxic group of anæmias, but not to the aplastic group. It is characterized by an abnormal production of red cells, because lead inhibits the incorporation of iron in the hæmatoporphyrin ring. The pathological production of erythrocytes and the synthesis of hæmoglobin may be recognized chemically by finding porphyrin III in excretions, morphologically by punctate basophilia, and functionally by the increased fragility of the red cells (hæmolysis). Sternal marrow usually shows a slight increase of normoblasts of all ages, occasionally also of proerythroblasts. Anæmia of lead poisoning at this stage responds well to liver preparations, and hæmatoporphy- [illegible]

relatively late according to our observations.

Anæmia due to Benzol and its Derivatives

This intoxication, which acts mainly by damaging the bone marrow, has received increased attention during the last few years It occurs in many branches of industry and especially in the leather and shoe trades, rubber, linoleum, ammunition and explosives processes and vulcanizing plants, and it may produce severe consequences. Benzol and benzene act by damaging the marrow, their derivatives (phenylhydrazine, phenol, resorcin, hydroquinone, toluylenediamine) more by hæmolytic properties. Miyamoto (1937) in rabbits poisoned with benzol, observed damage mainly to erythropoiesis According to Mignolet (1939) and Schwarz and Teleky (1941) the red cell series is affected earlier than the myeloid series. Bruni (1934) and Mallory *et al* (1939) found hyperplasia of the marrow in the early stages, followed by gelatinous transformation Ponticaccia (1923) and Schllowa (1930, 1933) in rabbits subjected to benzol intoxication noted hyperplastic marrow. Orzechowski (1929) reported selective damage to granulocytopoiesis, Fontana (1921) a gelatinous marrow poor in leucocytes, and Schmidtmann *et al* (1939) found leucopenia without definite atrophy of the marrow In rats, which had received subcutaneous injections of benzol, Latta and Davies (1941) noted increase of the neutrophil polymorphs, and myelocytic hyperplasia of the marrow The leucocytes were apparently destroyed rapidly in marrow and blood Bakalos and Thaddea (1943) gave 0 2–2 ml of benzol per kilogram of body weight to four rabbits and found that while small doses did not produce definite changes, the larger doses caused a picture similar to agranulocytosis

In man, Ronchetti (1922), Vercellotti (1928), Brindeau (1931), Meyer (1931), Weil and Perlès (1940), reported pernicious-like anæmia, but Gall (1938), Pabst (1938), Penati and Vigliani (1938), Erf and Rhoads (1939), Henny (1939), Hunter (1939), Lamy, Kissel and Pierquin (1939), Mignolet (1939), Weil, Perlès and Seror (1940), Ramvad (1941), Stodtmeister (1941), Danysz (1943) and others found an aplastic anæmia, as we did also At the same time leucopenia and thrombocytopenia were observed in most cases by Erf and Rhoads, Weil *et al.*, Lamy *et al.*, Henny, Leitner, Gall, Stodtmeister, Bernard-Pichon (1943), Garnier and Cordier (1943) and others. While hyperplastic reactions never occur according to Weil and his colleagues, Anderson (1934), Mallory *et al.* (1939) and Stodtmeister (1941) report hyperplasia of the red, and Penati and Vigliani (1938) hyperplasia of the white cells. Penati and Vigliani classify marrow damage from benzol intoxication into four types :—

(1) aplastic anæmia with marrow aplasia and no parenchymatous cells (except some lymphocytes).
(2) aplastic anæmia with active marrow, but inhibition of maturation, resulting in undifferentiated cell elements.
(3) atypical aplastic anæmia with leucocytosis and shift to the left, showing also a leukæmia-like hyperplasia in the marrow, liver and spleen (myeloid metaplasia).
(4) cases with the clinical and morbid anatomical findings of myeloid or lymphatic leukæmia.

Perrin, Kissel and Pierquin (1941) in a series of women with benzol poisoning, distinguished two types :—

(1) slight damage with red cell figures of 3 5–4 millions, leucocyte figures of 5,000, eosinophilia, thrombocytopenia of 150,000, prolonged bleeding time and symptoms of headache, pallor, loss of appetite and sleep and tendency to menorrhagia
(2) severe, chronic poisoning with considerable anæmia (R B C. 2 millions, Hb 20%–40%), anisocytosis, poikilocytosis, polychromasia, low colour index, leucopenia of 1,500–2,000 with granulopenia and thrombocytopenia of 50–100,000.

These authors maintain that marrow findings are important for assessing prognosis, but they did not find any marked hyperplasia of the marrow. Tzanck, Dreyfuss and Jais (1937), Gall (1938) Weil *et al* (1940) and Stodtmeister (1941) observed severe inhibition of maturation with 60% stem cells and up to 75% non-granular cells Duvoir, Dérobert and Albahary (1943) noted agranulocytosis with many primitive cells, which were also found in bone marrow, spleen and lymph glands Bakalos and Thaddea (1943) consider that hyperplastic reactions in benzol poisoning are

important for the understanding of the relationships between myelopathies and leukæmias.

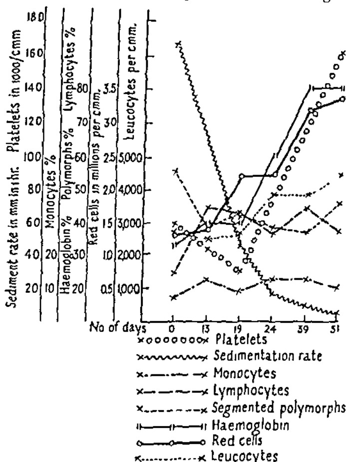
Fellinger (1938) could not find any damage to erythropoiesis in five moderately severe cases examined by sternal puncture. Weil *et al.* (1938), Perrin, Kissel and Pierquin (1941), Leitner (1941) and Bernard-Pichon (1943) believe that hæmatological control of workers exposed to the danger of benzol poisoning in industry is of great importance in early diagnosis. According to Goldwater (1941) macrocytic anæmia and thrombocytopenia are relatively frequent, but leucopenia is rare. Danysz (1943) did not observe neutropenia which is usually regarded as an early sign Garnier and Cordier (1943) described 2 cases with a monocytic-lymphocytic reaction and neutropenia, and Bernard (1943) reported absolute, as distinct from relative, lymphocytosis.

In most cases the blood picture returned to normal once the exposure had ceased, but Bénard, Poumailloux and Tiret (1943) reported a case in which a severe hæmopathy did not set in until twenty months after the cessation of exposure to benzol vapour. It is clear that onset and type of damage may vary considerably, apparently varying from case to case, but most authors agree that the damaging influence acts almost selectively on the bone marrow. According to Heim de Balsac, Perrault and Roubinet (1940) lack of Vitamin C predisposes to benzol poisoning ; according to Fail (1941) poor diet and starvation act similarly. According to Weil *et al* (1938) and Perrin *et al* (1941), women are especially liable to damage from benzol. Weil, Perlès and Aschkenasy (1938) conducted serial examinations of 50 persons engaged in the impregnation of clothing with rubber. They found anæmia in 33% of 33 men, whereas 82% of 17 women were anæmic Hypochlorhydria was common and 9 had achlorhydria. As 8 of these 9 workers eventually developed anæmia, it is likely that erythropoiesis becomes impaired secondarily to the damage to the gastric secretion. Two out of four children exposed to benzol were anæmic and one also had leucopenia and thrombocytopenia. The marrow was hypoplastic, and the anæmia refractory to all therapy. Previously undertaken serial examinations by Meyer and Schneider (1933) and Adler-Herzmark (1933) gave similar results, but they did not examine the bone marrow Hunter (1939) collected 89 cases of chronic benzol poisoning, only 3 of which had had a sternal puncture Leucopenia was not invariably present, but in 64% of the cases at least two cell systems were affected , only 12·4% had a normal blood picture Thirty-nine cases were anæmic and of those 35% died. He did not find that the female sex was more readily affected.

Decompensation of the marrow may reveal itself long after

(1938) warns against starting such work too early in life in view of the severity of the marrow damage. Other investigators (Weil *et al*, 1938) demand protective measures, Leitner (1941) and Danysz (1943) suggest change of work, while Bernard-Pichon (1943) recommends the elimination of benzol from industry.

Griva and Vigliani (1937) found blood transfusions without effect, Debray *et al.* (1941) gave pentnucleotide with good results.



GRAPH 10 Toxic anæmia (panmyelopathy) from chronic benzol poisoning Anæmia, leucopenia and thrombocytopenia Improvement of all values and fall of sedimentation rate from 160 mm to 5 mm after intensive therapy over 7 weeks.

We have observed recovery with iron and liver and stomach preparations as in the following case :—

Case 15. A man of 42 years, employed in a tinfoil factory, had worked with benzene for the last eighteen years Benzene is used for the moistening and flattening of thin tin foil The patient did not tolerate benzene vapour well and suffered from lassitude, headache, and occasional attacks of vomiting Many colleagues had similar complaints Eventually the patie [illegible] for going home [illegible] his passed off [illegible] ..ute work

After a slight injury he developed two abscesses in the gluteal region,

C.I 1·22; W.B.C.
polymorphs 55%,
ion of neutrophils,
aniso-macrocytosis, polychromasia; platelets, 25,000; sedimentation rate (Westergren) 166–174–177 mm. (1, 2 and 3 hr.), serum bilirubin direct and indirect, negative; Takata-Ara reaction negative, blood cholesterol 67 mg.%, blood urea 52 mg %.

STERNAL MARROW Early basophilic normoblasts 0 25, late normo-

among 400 cells.

There was thus a low figure for erythroblasts and a slight myelocytic-metamyelocytic shift to the left, but no marked disturbance of the maturation of the white cells. In spite of the hypoplasia of the marrow the disease (Graph 10) with Campolon, desiccated stomach, ferrostabil and arsylene (allyl arsenic acid) and the patient was discharged relieved.

In the sternal marrow the individual cell systems showed differing reactions; erythropoiesis was definitely hypoplastic, the more mature, predominantly orthochromatic normoblasts being the most numerous. Thrombopoiesis showed aplasia, while the granulocytic system, apart from the generalized reduction in cellularity and a slight shift to the left, was relatively unaffected The aplasia of all types of cells in the peripheral blood did not correspond to a panmyelophthisic state of the marrow, which, apart from the virtual disappearance of platelet precursors, showed only slight damage of the other cell systems. Mignolet's (1939) statement that anæmia is the first sign of benzol poisoning, and that leucopenia occurs only in severe cases, and that thrombocytopenia is a grave danger signal, are therefore not confirmed by our observations Anæmia should be regarded as an important early sign, and that is why benzol poisoning is being discussed with the anæmias. Damage to granulocytopoiesis and to thrombocytopoiesis does not necessarily follow in any particular order, nor does it depend on the severity of the intoxication, but may occur at the same time, or even may occur as the initial sign. Hæmolysis was excluded as a causal factor in our cases as the serum contained no excess of bilirubin It is interesting to note that polycythæmia may occur in benzol poisoning (Mallory *et al*, 1939) Mondon and André (1941) reported 2 cases with red cell counts of over 7 millions.

Summary. Benzol has a definitely toxic action on bone marrow, but women appear to be affected rather more severely than men It is not certain whether the menstrual loss of blood

favours anæmia from benzol poisoning. It is probable that constitutional factors play an important part, because as a rule only a certain number of the workers exposed to risk become affected. The damage to the gastric mucosa appears to be important, as observations show that anæmia is rather more frequent in subjects with achlorhydria. Benzol derivatives (such as phenol, phenylhydrazine and others) cause hæmolysis, but they are seldom if ever toxic to the bone marrow.

Because benzol causes very severe damage to the marrow, often with fatal results, the state of health of the industrial workers must be safeguarded by repeated blood counts and prophylactic measures. Serial blood counts on workers with benzol have the same importance for early diagnosis as serial X-ray examinations in those in danger of tuberculosis.

Other Toxic Anæmias

Several therapeutic agents may at times have a hæmotoxic action. Among the metallic substances, gold is used widely in the treatment of tuberculosis and rheumatoid arthritis and Halberkann (1935), Hulst (1937), Weil, Oumansky and Langlois (1938) and Wintrobe *et al.* (1939) have observed anæmia following gold therapy, sometimes of an aplastic type In two rabbits Lefèvre (1937) noticed anæmia after the administration of gold. We (Leitner, 1938) have collected a series of 53 tuberculous patients, who had regular blood examinations during treatment with gold, but anæmia was not found nor was there any increase in reticulocytes. In 18 patients we performed sternal puncture, but found no evidence of marrow damage. We have shown by histochemical methods that gold is deposited in the marrow of rabbits treated with it and damage to hæmopoiesis is thus quite feasible

Willi (1943) has reported an acute hæmolytic anæmia with the formation of methæmoglobin and intracorpuscular bodies in an infant after treatment with Anastil (a preparation of guaiacol) Schilling (1933) noted anæmia with intracorpuscular bodies and Meulengracht and Lundsteen (1939) observed 11 cases with anæmia and cyanosis in acetanilide poisoning, while Engelbreth-Holm examined the marrow in this series and found hyperplasia of all three cell systems

Alder (1937) reports a case of polycythæmia treated with benzol, phenylhydrazine and X-rays which became transformed into an aplastic anæmia with hæmoglobin 27% = 4·4 g %, leucocytes 2,200 and platelets 40,000 per cmm Whitby and Britton (1946) have encountered a similar case Neosalvarsan also, which contains a benzol ring, may damage leucopoiesis, but various authors have found a greater tendency to thrombocytopenia and aplastic anæmia rather than simple anæmia (Bamforth and Elkington, 1931,

Corelli, 1936; Kadin, 1938, Levin and Keddie, 1942; and Dubois-Ferrière and Jeanneret, 1943). In a case of stovarsol poisoning, Smith and Lyon (1935) found the tibial marrow aplastic.

Following amidopyrine, Rohr (1940) noted anæmia and Heubner (1941) found anæmia with intracorpuscular bodies after maretin (m-tolyl-hydrazino carbaminate).

As already mentioned, phenylhydrazine, resorcin, hydroquinone, tyrosin, tyramin, toluylenediamine and trichlorethylene may cause hæmolysis and do not primarily attack hæmopoiesis, as shown by Ingrassia (1933), Baldridge (1935), Gunther (1935), Manzine (1936), Campanacci and Falzoy (1937) and Stock (1937). According to Rhoads and Miller (1938) saponin interferes with marrow regeneration.

Anæmias from sulphanilamide and allied preparations especially are of topical interest. Sulphanilamide preparations not only have a hæmolytic action, but are also generally toxic to marrow (see chapter on agranulocytosis) Hæmolytic anæmias have been described by Harvey and Janeway (1937), Myhre (1938), Wood (1938), Fox and Ottenberg (1941) and Glanzmann (1942) Trier (1941) collected 90 cases from the literature and reported a case of his own, and this indicates the frequency of anæmia caused by these drugs Paul and Limarzi (1940) reported a case of hæmolytic anæmia from the administration of sulphanilamide, in which sternal marrow showed normoblastic hyperplasia with early erythroblasts. Moeschlin (1940) observed anæmias with intracorpuscular (Heinz-Ehrlich) bodies which occurred on the fourth to sixth day of treatment with sulphapyridine. In sternal marrow, which showed hyperplasia of normoblasts, he found fewer intra-corpuscular bodies, but increased numbers of reticulocytes compared with the peripheral blood. The reticulocytes only rarely contained intracorpuscular bodies Moeschlin believes that this indicates the peripheral origin of the intracorpuscular bodies of Heinz-Ehrlich following methæmoglobinæmia. Heubner (1941) believes that methæmoglobinæmia, being a reversible process, does not lead to the formation of intracorpuscular bodies nor does it cause destruction of red cells and therefore anæmia. Poisons which produce methæmoglobin can cause extensive oxidation of hæmoglobin, which may be irreversible and may cause in turn decomposition of hæmoglobin and destruction of the erythrocytes These oxidative processes cause a break up of the hæmatoporphyrin ring and verdohæmochromogen is formed Their lines of absorption in the spectrum may be confused with those of methæmoglobin or may be mistaken for sulphhæmoglobin. Further oxidations may attack the albuminous portion of hæmoglobin and cause reduced solubility and a higher intensity of staining with the dyes He considers this is the cause of the formation of intracorpuscular bodies. Those poisons which lead to the formation of intracorpuscular bodies

in intoxications with fluorine, lead, arsenic or phosphorus, which may lead to marrow hypoplasia.

Markoff believes that the marrow changes are the primary process. Marrow hypoplasia is followed by an increased osteoblastic activity of the endosteum, so that osteosclerosis is produced on a myelogenous basis Apitz (1940) holds the view that an inflammatory atrophy of the marrow is the primary process. Some toxin causes increased permeability of the marrow blood vessels, and erythrocytes and fibrin reach the parenchyma and cause its transformation into fibrous marrow. Clairmont and Schinz (1924) have recognized the primary importance of the marrow changes in the development of osteosclerosis in the adult, but Schmidt (1927) believed that the bone and the marrow changes run parallel

Not every marrow atrophy, however, will lead to osteosclerosis In myelosclerosis of the type described by Vaughan (1936) in which Vaughan and Harrison (1939) have observed marrow fibrosis in histological sections, the radiological picture does not show typical osteosclerosis, but only an irregular thickening of the spongy layer and a decreased thickness of the cortex especially in the long bones In this myelosclerosis or myelofibrosis as it is better called, splenomegaly is common, as is also a leuco-erythroblastic anæmia with 3%–12% erythroblasts and scanty myelocytes present in the peripheral blood It is, therefore, likely that some special stimulus or a constitutional element must be at work in order to produce osteosclerosis

Only a few sternal punctures have been performed in osteosclerosis Binder and Riedl (1942) reported 2 cases of osteosclerotic anæmia, in which the diagnosis was made by sternal puncture owing to the hardness and thickness of the cortex. Weil and Perlès (1940), Heilmeyer (1942) and we ourselves have pointed out the diagnostic importance of the thickness of the cortex, but Frank and Breitkreuz (1944) found sternal puncture very easy in one case and yet recorded an aplastic marrow picture. It is possible that the osteosclerosis was not generalized or probably it was a case of myelofibrosis. We have on occasions observed thickening of the cortex in chronic myeloid or in lymphatic leukæmia Recently we (Leitner, 1944) have reported a case of secondary osteosclerosis in the presence of hyperadrenalism, in which sternal puncture proved difficult owing to the thickness of the cortex Sternal punctures may thus aid the diagnosis of osteosclerosis Whitby and Britton (1946) stress the need for differentiation between myelofibrosis where the bone marrow is replaced by fibrous tissue and the bone itself may be thinned rather than thickened, and myelosclerosis where the bone marrow is replaced chiefly by bony tissue and the bone is thickened In their cases of myelofibrosis and those of Rosenthal and Erf (1943), and Erf and Herbut (1944), the bone marrow showed fibrosis with numerous megakaryocytes and only

a few small islands of erythropoietic and leucopoietic tissue. The greater proportion of hæmopoiesis takes place in extra-medullary sites, especially in the enlarged spleen, in the retroperitoneal tissue or even in the psoas muscle. The marrow picture is best seen when sections are cut of trephined material from the sternum. Certain cases of this type have been described under the title of *Agnogenic Myeloid Metaplasia of the Spleen*. The bone marrow is described as aplastic or fibrotic, and is rarely hyperplastic, and never suggestive of leukæmia. The peripheral blood presents a leukæmoid picture, though the total cell count is not usually increased. Jackson *et al.* (1940), Levinson and Limarzi (1947) and Heller *et al.* (1947) pointed out the marked extramedullary hæmopoiesis in the spleen and occasionally in other organs. This process is almost always confined to the myeloid series of cells. Reich and Rumsey (1942) state that it is easy to confuse splenic neutropenia with agnogenic myeloid metaplasia and advise caution. When splenectomy or irradiation to the splenic area was carried out in cases of metaplasia, the disease took a turn for the worse and often terminated like myeloid leukæmia.

HÆMOLYTIC ANÆMIAS

Numerous classifications of the hæmolytic anæmias have been suggested, but their discussion would lead too far afield. For our purposes the most convenient grouping is the following :—

Acquired Forms. (1) Acute hæmolytic anæmia of Lederer (Lederer-Brill). (2) Paroxysmal nocturnal hæmoglobinuria (Marchiafava-Micheli). (3) Acquired hæmolytic jaundice and anæmia with splenomegaly.

Constitutional Forms (1) Familial hæmolytic icterus (spherocytic anæmia, Minkowski and Chauffard). (2) Familial elliptocytosis (anæmia of ovalocytosis, Dresbach). (3) Sickle cell anæmia (African anæmia, drepanocytic anæmia, Herrick) (4) Erythroblastic anæmia (Mediterranean anæmia, Cooley)

Anæmia of the Neo-natal Period and Infancy (1) Hæmolytic anæmia of the new-born (erythroblastosis fœtalis, Rautmann, v Gierke) (2) Anæmia of prematurity. (3) Constitutional infantile pernicious-like anæmia (Fanconi) (4) Pseudo-leukæmic anæmia of children (v. Jaksch-Hayem-Luzet)

The acute erythræmic myelosis of di Guglielmo, does not belong to this group. The same applies to chronic forms of erythræmic myelosis, *e.g.*, type Heilmeyer-Schöner Unfortunately there is great confusion in classification and nomenclature, where these rarer forms are concerned Both diseases have been called erythroblastosis by various authors, and have been grouped among others with Cooley's anæmia. We have attempted to separate the malignant systemic diseases of the erythroid series, which correspond

more to the leukæmias, from the hæmolytic anæmias. As to terminology we follow di Guglielmo's suggestion and speak of acute or chronic erythræmic myelosis. There is some possibility of confusion in this classification, as French and American authors often use the name erythræmia for polycythæmia. But the use of erythræmic as an adjective (erythræmic myelosis) makes distinction possible. By erythroblastosis is meant the presence of erythroblasts in appreciable numbers in the peripheral blood and more specifically this applies to Cooley's anæmia and to the erythroblastic anæmias of the new-born.

Acute Hæmolytic Anæmia (Lederer-Brill)

This is really an anæmia due to an infection of unknown cause, with pyrexia, headaches, pains in the limbs, enlargement of the spleen and liver, jaundice and high leucocytosis (up to 100,000 per cmm.) although leucocytosis may be absent (Giordano and Blum, 1937; Dameshek and Schwartz, 1940), macrocytosis with occasional tendency to micro-spherocytosis and frequently bilirubinæmia and urobilinogen in the urine. According to Lederer (1930) the infection has a selective action on the reticulo-endothelial system. Dameshek and Schwartz (1940) and Currie (1944) have described cases in which true red cell hæmolysins were present, which could be neutralized by adding normal human serum. This may explain the favourable effect of blood transfusions in these cases. Naegeli (1935) as well as Heilmeyer (1939) consider that some constitutional factor may play a part Fanconi (1939) reported the case of a patient aged eleven, which presented features of both familial hæmolytic icterus and Lederer's anæmia. In Heilmeyer's case microspherocytosis only disappeared three and a half years after the case came under observation. Goudsmit (1935), Debré, Lamy and Bernard (1937), Greenwald (1938), Glanzmann (1943) and Heilmeyer found erythrocyte resistance normal or reduced

Because, as Glanzmann has observed, there are erythroblasts, leucocytosis and immature white cells in the blood picture, there is a possibility of confusing this disease with leukæmia Prompt diagnosis is most important, because repeated blood transfusions often have a very striking therapeutic effect This has been confirmed by Lederer (1930), Giordano and Blum (1937), Greenwald (1938), Heilmeyer (1939), Glanzmann (1943) Meier (1944) and others In the determination of blood groups, panagglutination or autoagglutination may be troublesome as reported by Gsell (1945) If transfusions fail, splenectomy should be carried out (Dameshek and Schwartz, 1940) The disease is a rare one and Giordano and Blum (1937) collected only 52 cases from the literature

Meier (1944) found an increase in the sternal marrow of erythroblasts (79 per 100 white cells, and in a second case, 40 5 per 100),

in which proerythroblasts, early and late normoblasts took part. Gsell's case showed 173 normoblasts per 100 white cells Bonell (1941, 1942) reported an increase of normoblasts, hyperplasia of the white series with eosinophilia and plasma cell hyperplasia, and he concluded that this might indicate an allergic origin, especially as Luisada (1941) in a case of favism (hypersensitivity against fava beans) reported similar marrow findings Glanzmann (1943) and Meier (1944) in 1 case observed eosinophilia in the marrow, and attribute some part in the ætiology to hypersensitivity against plums Spira (1943) doubted if Bonell's case was a true case of Lederer's anæmia. Our own impression is that the pronounced leucocytosis favours the theory of an infectious ætiology. Tischendorf (1939) reports a case very similar to Lederer's anæmia, in which the sternal marrow showed normoblastic hyperplasia and the peripheral blood showed a myeloid leukæmoid reaction Giordano and Blum (1937) at autopsy found marrow hyperplasia, hæmosiderosis, and focal necrosis in the liver

Paroxysmal Nocturnal Hæmoglobinuria (Marchiafava-Micheli)

This disease is characterised by marked hæmolytic anæmia and is distinguished from paroxysmal (cold) hæmoglobinuria by its nocturnal hæmolysis and by the absence of cold hæmolysins, *i e*, by a negative Donath-Landsteiner test Donath and Landsteiner (1905) showed that the hæmolysis in paroxysmal (cold) hæmoglobinuria was due to a hæmolysin in the patient's blood which unites with the red cells when the temperature is low; when the temperature rises the amboceptor-sensitized cells become lysed by the complement normally present in the serum

In paroxysmal nocturnal hæmoglobinuria the amboceptor is contained in the red cells and a thermolabile complement which is alleged to be present in normal serum as well as in the serum of patients is necessary to cause hæmolysis When the blood is kept in the incubator autohæmolysis occurs after 15–60 minutes, depending *on the severity of the case, as the oxygen tension in the blood* falls and that of the carbon dioxide rises (Jordan, 1938, Ham, 1939, Buell and Mettier, 1941, Hegglin and Maier, 1943) In a case described by Heilmeyer and Wengeler (1943) the red cells were found to be abnormal while in a second case the serum appeared to be at fault Dacie, Israels and Wilkinson (1938), Ham (1939), Hegglin and Maier (1943), Heilmeyer and Wengeler (1943) found hæmolysis increased when the *p*H of the blood was lowered During the night, owing to a vagal effect the serum *p*H shifts towards the acid side and thus the nocturnal character of hæmoglobinuria becomes intelligible Ham and Horack (1941) have based their acid serum test for this disease on this fact.

Hæmoglobinæmia and hæmosiderinuria persist, but hæmoglobinuria disappears during the day. In the case reported by Dacie *et al.* there was spherocytosis (spherical index 0·43), while erythrocyte resistance was not reduced, while in the case of Abicht *et al.* (1943) the erythrocyte resistance showed a definite decrease. This serious disease is very rare fortunately, as it is refractory to all forms of treatment, and splenectomy is useless. Some 56 cases have so far been reported in the literature, which is reviewed by Hamburger and Bernstein (1936), Dacie *et al.* (1938), Hoffman and Kracke (1943) and Manchester (1945).

In sternal marrow, Brulé, Hillemand and Gaube (1938) found normoblastic hyperplasia; Buell and Mettier hyperplasia of leucopoiesis and erythropoiesis with primitive normoblasts; Scott, Robb-Smith and Scowen (1938) a simple normoblastic reaction, and Heilmeyer and Wengeler considerable normoblastic increase with 142 nucleated red per 100 white cells (20 early normoblasts). Hegglin and Maier observed a large increase of normoblasts just as in hæmolytic jaundice; figures for reticulocytes were higher in the marrow than in the peripheral blood, and there were numerous mitoses as well as karyorrhexis.

Acquired Hæmolytic Jaundice and Anæmias with Splenomegaly

The ætiology of these pathological conditions is still a matter of discussion. Naegeli (1931), Gänsslen (1936), and others do not admit the possibility of acquiring spherocytosis or hæmolytic jaundice On the other hand, Heilmeyer (1939) believes that spherocytosis and a lowered resistance of erythrocytes are an expression of some functional mechanism and may be acquired As the cause of this acquired hæmolytic condition, Weber and Bode (1932) and Hoff (1934) have suggested chronic infections, such as syphilis, König (1924) and Curschmann (1930) have suggested malaria, Chalier (1933), Loeper *et al.* (1941), Singer and Dameshek (1941), Jones and Tillman (1945), and Stats *et el.* (1947) various tumours. Leitner (1935), Weil and Perlès (1938) and Kjerulf-Jensen (1943) mention tuberculosis. Davis (1944), who describes 4 cases, suggests that the underlying disease may sometimes stimulate the reticulo endothelial system to abnormal activity. Removal of the cause often leads to dramatic recovery Heilmeyer (1935) expresses the opinion that the spleen produces some factor, which causes erythrocytes to become more spherical and less resistant Frank (1925) suspects an inhibition of marrow function by this splenic factor, Lauda (1937) suggests inhibition of cell production and Bucheim (1939) an inhibition of the release mechanism of cells from the marrow We believe that an inhibition of cell maturation is present According to Guichard and Jeune (1942) increased hæmolysis and

phagocytosis of erythrocytes in the spleen are of importance. In our opinion the splenic inhibition of the marrow and the increased hæmolysis are often due to hyperfunction of the reticulo-endothelial system of the spleen. It is probable that the hæmolytic anæmias described by Greppi (1938) and a certain number of cases termed Banti's Syndrome also belong to these "hæmolytic, hypersplenic" states. Wiseman and Doan (1942) and Doan and Wright (1946) describe a condition of primary splenic panhæmatopenia, which they believe is due to splenic hyperfunction. They postulate a number of groups in which the spleen destroys chiefly, either neutrophils (primary splenic neutropenia), or red cells (hæmolytic anæmia), or platelets (thrombocytopenic purpura). Splenectomy in their hands is often dramatically curative. In Ferrar, Burneth and Steigman's case (1940) splenectomy resulted in cure, but Heilmeyer and Albus (1935) hesitate to advise operation because the anæmia tends towards a spontaneous cure Hoff (1934) achieved good success with potassium iodide. Recently Loutit and Mollison (1946) have shown that washed red cells from cases of acquired hæmolytic jaundice are agglutinated by rabbit anti-human globulin serum, whereas those of familial hæmolytic jaundice are not. Thus they state that in the acquired form an abnormal "hæmolysin-cohæmolysin" system is in action

Bone marrow of the cases investigated by sternal puncture is usually similar to the congenital type and shows an increase in normoblasts, as reported by Mallarmé (1937), Fieschi (1940), and

any reduction of megakaryocytes. In different cases Mallarmé found hyperplasia normal figures and sometimes hypoplasia of the marrow

HÆMOLYTIC ANÆMIAS DUE POSSIBLY TO A CONGENITAL ANOMALY OF THE ERYTHRON

Familial Hæmolytic Icterus (Minkowski–Chauffard)

This disease is the hæmolytic anæmia which has been most extensively investigated In 1900 Minkowski discovered the hereditary nature of this jaundice which is invariably accompanied by splenomegaly In 1909 Chauffard and Vincent observed the increased fragility of the erythrocytes and Alder (1927) and Naegeli (1931) noted their spherical shape ("anæmia of spherocytosis") The spherical index according to Heilmeyer (1939) is 0 6 instead of the normal 0 3. We share the view of Gansslen (1936), Haden (1940) and others, that the spherical form is the most important cause of the hæmolysis, because the cell envelope of the spherocyte,

which has a volume of 100 c μ instead of the 78–94 c μ of the normocyte, is under greater pressure than the cell envelope of the latter. It is still uncertain whether the overactivity of the spleen is the primary lesion, as suggested by Heilmeyer and Albus (1935). In favour of a peripheral causation is the fact that reticulocytes are not microspherocytes, but normal in size. Dameshek and Schwartz (1938) have produced spherocytosis in the peripheral blood of experimental animals by injecting serum rich in hæmolysin. Willenegger (1944) obtained evidence of hypersplenism in a Group A female patient who had been transfused with Group 0 blood 17 hours prior to splenectomy. The patient's erythrocytes were lysed by Group A specific hæmolysin, and smears from the splenic pulp were examined These showed evidence of degeneration of the transfused erythrocytes, a feature which was absent in the peripheral blood. Dacie and Mollison (1943), however, found that red cells from a case of congenital hæmolytic icterus, both before and after splenectomy, were destroyed at a greatly increased rate when transfused into normal persons, whereas normal cells survived for the normal time when transfused into patients with the disease. It is possible that spherocytosis, just as is the case with the elongated forms in elliptocytosis (p 155), is a manifestation of senescence of the cells, the primary cause of which may be maldevelopment of the red cells in bone marrow Owing to the severe hæmolysis, jaundice often develops as well as splenomegaly, a rise in indirect bilirubin, and eventually anæmia, with the well-known symptoms of lassitude, giddiness, headache, noises in the ears and shortness of breath Urobilin, urobilinogen and uroerythrin are present in the urine The colour index is usually about unity In saline solutions, hæmolysis begins at 0 52%–0 64% NaCl instead of the normal 0·44% NaCl.

In spite of much increased destruction of erythrocytes anæmia does not always become severe This must be due to an increased production of erythrocytes We have even seen cases without anæmia In the blood picture the increased erythropoiesis is expressed by a considerable increase of reticulocytes, in which the juvenile forms participate All authorities are agreed that the sternal marrow shows a pronounced increase of erythroblasts, chiefly normoblasts, but the proerythroblasts are also increased Even before the introduction of sternal puncture, Micheli (1911), Ginzetti (1912) Sisto (1914), and Eppinger (1914) had recorded an increase in normoblasts This finding was confirmed later by trephine biopsy Weiner and Kaznelson (1926) observed up to 60% erythroblasts Escudero and Varela (1927), who used to trephine the tibia, found twice as many erythroblasts as granulocytes Dameshek (1935) reported similarly

Sternal puncture gave corresponding results Rohr (1940) found sometimes more than 100 erythroblasts per 100 white

cells, Henning and Keilhack (1939) up to 70%, Klima (1938), Schwarz (1928), Leitner (1941) and Thaddea and Bakalos (1940) reported similar figures; Markoff (1936) found 39% De Weerdt (1938) in 7 cases found between 100 and 300 erythroblasts per 100 white cells, Tøttermann (1936) up to 82%, Löwinger (1936) up to 50%, Piney (1933) 45%–64% (25%–34% of which were proerythroblasts) Mallarmé (1937) found the proportion of red to white cells was altered from 2 : 3 to 1 · 3, but found no difference in his figures in remissions or exacerbations Fieschi (1940) in 1 case found an erythroid-myeloid ratio of 92 · 8, and in another, of 45 : 55 According to de Weerdt, Klima, Rohr, Markoff, Fieschi, Heilmeyer and our own experience also, the hyperplasia extends to the proerythroblasts and early normoblasts. (Fig 108)

Schulten (1939) found an increase of micronormoblasts and of macronormoblasts; Picena (1937) of orthochromatic normoblasts;

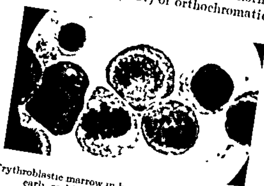


Fig 108 Erythroblastic marrow in hæmolytic jaundice (proerythroblasts, early and late normoblasts) (× 1,050)

Oria, Ramos and Tranchesi (1938) of micronormoblasts, but Henstell and Dameshek (1936) found normal sized normoblasts In a hæmolytic crisis the proerythroblasts are increased and they may be very large, as reported by Thaddea (1943) Fieschi (1940) interprets this phenomenon as an inhibition of regeneration, whereas mature normoblasts indicate satisfactory regeneration. We have only examined one case during a crisis and found an enormous hyperplasia of erythropoiesis with 12 proerythroblasts, 80 early and 200 late normoblasts per 100 white cells and the more primitive forms predominated An atypical finding was recorded by Tøttermann (1936) who noted megaloblasts following splenectomy In 1 case we have observed a fall in the number of erythroblasts after splenectomy and Löwinger (1936), Schartum-Hansen (1937) and Thaddea (1943) report similar findings According to Löwinger it is only the macronormoblasts and not the micronormoblasts which are reduced We have not seen megaloblasts in this disease and therefore presume that reports of megaloblasts in this disease are due to differences in the definition of this cell Dameshek (1935), Markoff

(1936), Löwinger (1936), de Weerdt (1938) and Fieschi (1940) point out the large numbers of mitoses which we also noticed. *Fieschi found* 4 0%–5 0%, Markoff 5%, Löwinger 8%, de Weerdt 3·7% (instead of 2·72%) of the erythroblasts in karyokinesis. In 1 case we noted 4·6% and in another 3·3%.

Ungricht (1938) examined the reticulocytes and found that hæmolytic jaundice was the only condition in which there were fewer reticulocytes in the marrow than in the peripheral blood and we have confirmed this observation. Prokowsky (1930), Markoff (*1936*), de Weerdt (*1938*) and Klima (*1938*), however, found higher marrow reticulocyte counts just as in other diseases. Ungricht explains this discrepancy by the suggestion that reticulocytes in peripheral blood with only a single supravital granule might have been missed. The persistence of these forms, according to Seyfarth (1927), is due to some disturbance of maturation of the reticulocytes. Schartum-Hansen (1937) investigated figures of reticulocytes before and after splenectomy and found that the differences between reticulocyte counts in marrow and in peripheral blood became smaller, the nearer erythropoiesis returned towards normal. The following case shows how sternal puncture may confirm the diagnosis even in those cases where the anæmia is not marked and the jaundice only slight :—

Case 16. B W., a boy of 13, was admitted to hospital with a sore throat and high temperature. When examined, *he had an inflamed* throat and a suggestion of jaundice , the spleen was one finger's breadth below the costal margin and the edge was firm. The patient's mother also had hæmolytic jaundice

BLOOD. *R B C 3·6 millions, Hb* 74·4% = 12 0 *g.*%, C I. 1·03; [illegible] forms 2%, segmented
Anisomicrocytosis
[illegible] and 2 hr.) Serum
[illegible]
[illegible] ly normoblasts 5 5,
[illegible]
semimature myelocyte [illegible]
10·5%, stab forms [illegible]
myelocytes 3 5%, [illegible]
lymphocytes 7 5%, [illegible] aryo-
cytes 1%, plasma c[illegible] cells
1 5%. Reticulocytes in the marrow 2·2%, in the blood 3·7%.

There was thus a considerable erythroblastic reaction in which proerythroblasts took part (see Fig 108) There was also a myelocytic shift to the left which agrees with the observations of Markoff (*1936*), de Weerdt (1938), Greif (1938) and Thaddea and Bakalos (1940) There could have been no question of a reaction to infection in this case, because sternal puncture was not performed until the sore throat had subsided.

Summary. Hæmolytic icterus is *accompanied by a more or* less definite increase of normoblasts in the sternal marrow Only

very few reports of normal erythropoiesis exist, such as by Roversi and Tanturri (1935), who found only 24·21% erythroblasts in a single case, and by Oria, Ramos and Tranchesi (1938), who found only 25 nucleated red cells per 100 white cells. The diagnosis can normally be made on clinical grounds with jaundice, abnormality of the cranial bones, splenomegaly and by the hæmatological findings, such as microspherocytosis, reticulocytosis and increased red cell fragility. It is worth remembering that this increased red cell fragility may be masked, and therefore Wiedemann (1942) advocated repeated fragility tests, possibly with washed red cells and after contracting the spleen by an injection of adrenalin. Wiedemann recorded five members of a family with hæmolytic jaundice, in whom hæmolysis commenced at the normal saline concentration. The increased maximal resistance and reduced breadth of the resistance curve were the only signs of the increased red cell fragility.

Sternal puncture reveals a definite erythroblastic hyperplasia, which varies according to the degree of anæmia and the degree of red cell destruction. This in turn varies with exacerbation or remission. Hyperplasia of erythroblasts, mainly of early normoblasts and proerythroblasts, often helps to establish the diagnosis of hæmolytic icterus. The increased number of mitoses of the red series, and a slight shift to the left of the white series, are characteristic. Following splenectomy the marrow usually returns to normal. Certain hæmatological findings may disappear such as microspherocytosis as reported by Dominici (1901), Doan, Curtis and Wiseman (1935), Momigliano-Levi and Bairati (1935) and Heilmeyer (1939). Hawksley and Bailey (1934) reported their transient disappearance, while Hawksley (1936) and Mogensen (1938) found no alteration in the microspherocytosis after splenectomy.

Ovalocytosis (Familial Elliptocytosis)

This disorder was discovered in 1904 by the American physiologist Dresbach. Huck and Bigalow (1923) were the first to demonstrate the familial trait of ovalocytosis. The phenomenon is inherited as a simple Mendelian dominant so that males and females are equally affected and have equal powers of transmission, but unaffected subjects do not transmit (Strauss and Daland, 1937). Since then the attention of hæmatologists has been attracted by the anomaly and papers have been published in many countries. Bernhardt (1928) and van den Bergh and Rehorst (1931) reported the first cases in Europe. Including the two families recorded by us (Leitner, 1939, 1943) more than twenty-four families are known to be affected. Reviews of the condition have been published by Florman and Wintrobe (1938), Miller and Lucas (1938), Cooley (1942) and many others. It is important to realize that, as well as

Sickle Cell Anæmia (African Anæmia, Drepanocytic Anæmia)

While ovalocytosis is not a disease confined to certain r sickle cell anæmia, discovered in 1910 by Herrick, occurs ma in North American negroes, though it has very occasionally l *reported in white people, who had no trace of negro ance* (Brandau, 1930; Cooke and Mack, 1934; Haden and Ev 1937, Makrycostas, 1940; Greenwald and *Barret*, 19 Greenwald *et al*, 1943; and others)

Sickle cells occur in North American negroes in a latent fo according to Cooley and Lee (1929) in 7·5%, and Graham McCarty (1930) in 7·2%. Anæmia manifests itself only in 0 *according to Sydenstricker (1924), but the percentage is highe* other areas (Tomlinson, 1945) Once anæmia has appeared, disease becomes a very severe one. It is, *however, not as freque* fatal as was previously thought, and especially less so after thirtieth year of life The clinical symptoms are similar to th of hæmolytic icterus, that is jaundice which in negroes is o recognizable in the sclera, bilirubinæmia, and urobilin in urine, splenomegaly *followed frequently in the later stages* atrophy of the spleen from thrombotic occlusion of the vess *ulcers on the legs, skeletal changes with enlargement of the marr* cavity owing to marrow hyperplasia, crises with pains in the reg of liver and spleen. pyrexia, nausea and vomiting. Erythroc fragility is not increased and the red cells are normochromatic

The sickle shape of the cells is not usually found in ordina blood smears *In sealed moist preparations, however, the tenden* to sickling increases, and becomes definite in 4 hours, a maximal in 24 hours, when 90%–100% of the red cells a sickled While Josephs (1938) incriminates some factor in the seru (factor D) as the cause, Hahn and Gillespie (1927) proved th carbon dioxide favoured sickling, but oxygen opposed it Th sickling is a phenomenon following anoxæmia Hahn and Gillesp *state that an oxygen content of 6·4% at a partial pressure of 45 mi* Hg suffices to prevent sickling It should be pointed out th it is difficult to see how certain phenomena agree with this conce tion. American authors have achieved, even without saturati with oxygen, disappearance of the sickling of drepanocytes, whe they were suspended in normal serum *On the other hand sick* formation appeared when erythrocytes were suspended in a solu tion which *contained traces of serum from a case of drepanocytos*

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believes that because sickling temporarily disappears after splene tomy, as reported by Hahn and Gillespie (1927), such a factor may b of some importance *The resulting anæmia is resistant to therapy* Cures have not been observed even after splenectomy Digg

(1935) considered that atrophy of the spleen developing in the course of the disease may be "autosplenectomy."

Jaffé (1927) has found hyperplasia of erythropoiesis with round normoblasts in the marrow, while Venco and Fisher (1941) noted hæmorrhages and necrotic foci. In sternal marrow Makrycostas (1940) found an increase of proerythroblasts, early and late normoblasts and reticulocytes, as well as mitoses, but Sharp and Schleicher (1936) observed only a moderate increase of normoblasts. Mason (1922) reported hyperplasia of granulocytopoiesis also. Wintrobe (1946) states that the bone marrow may contain 50%–70% of nucleated red cells, mostly normoblasts, but with some immature forms. A few of the normoblasts may be of abnormal shape, but this is unusual. There may be a moderate shift to the left of the myeloid cells and an increase in eosinophils and megakaryocytes. Monocytes may be found to contain ingested red cells and nuclear fragments and frequently granules may be present.

Erythroblastic Anæmia (Cooley) (Thalassæmia, Mediterranean Anæmia, Target-oval Cell Syndrome)

This disease, discovered in 1925 by Cooley and Lee in U.S.A., is found chiefly in children of Italian and Greek immigrants. Its exact diagnosis and nature is still very controversial, but the consensus of modern opinion is that it is an anomaly of the red cells in Mediterranean peoples, somewhat comparable to the sickle cell anæmia of African negroes. The red cells are often thin and oval or target shaped.

Di Guglielmo (1928) regards this condition as a chronic form of his acute erythræmic myelosis, and therefore as a dyscrasia of the red cell series corresponding to leukæmia. Schiappoli (1939) and Colarizi and Biddau (1940), as well as marrow hyperplasia and hyperplasia of the reticulo-endothelial system in the marrow, observed evidence of much extramedullary hæmopoiesis in the liver and spleen. Differentiation from di Guglielmo's disease is therefore not always easy. The disease is not absolutely confined to inhabitants of the shores of the Mediterranean, for Bywaters (1938) has reported it in an English child of ten, Graser (1941) in a German child, Freudenberg and Esser (1942) in a Swiss child, Kohlbach (1941) in a German adolescent, and Panoff (1938) in two Bulgarian children. The inhabitants of the Mediterranean countries belong to various races, and thus also is a point against a pure racial origin, and with Pehu and Lenche (1940) we would prefer to consider it as due to similar living conditions. Choremis and Spiliopoulos (1937) suspected some relationship to malaria, but Marcolongo (1937) and others could not confirm this. In the Swiss and German cases no trace of infectious disease could be demonstrated. Because the disease manifests itself in the second year of life and leads to

death in a few years, the patients do not reach the age of *reproduction* (*vide infra*). Caminopetros (1938), Panoff (1938) and Atkinson (1939) found increased erythrocyte resistance in healthy parents, while Malamos and Delijannis (1940) found increased numbers of normoblasts in the sternal marrow of healthy parents and brothers *and sisters. Chini (1939) reported skeletal* and marrow changes, and Saracoglu (1943) anisopoikilocytosis. These findings may be regarded as latent signs of Cooley's *anæmia. Since the work of* Wintrobe *et al.* (1940), Smith (1943), Dameshek (1943), Valentine and Neel (1944) and Neel and Valentine (1945) it has become clear that there are latent and overt cases of the disease Genetic studies suggest that homozygous individuals, that is those who inherit the "thin red *cell*" or "target *cell*" trait *from* both parents, manifest the complete disease, whereas heterozygotes exhibit only mild *forms.* The *fact that the complete disease is almost always fatal in* childhood explains why adults usually exhibit no more than slight or symptomless forms. Since the characteristic target cells are thinner than normal, that is the reverse of spherocytes, they tend to show increased resistance to saline hæmolysis. The characteristic target *cell* picture is also described by Schieber (1945), Greenblatt *et al.* (1946) and others. Clinically the disease is *characterized by anæmia, extreme hepatomegaly and spleno-* [illegible] with a subicteric [illegible] blood is a considerable erythroblastosis with many immature and abnormal forms and with a tendency *to lobation of the nuclei, and variation* in size. Lehndorff (1936), therefore, coined the term "*para-erythroblasts*" *for these* cells. There is gross anisocytosis poikilocytosis and punctate basophilia with the presence of target and oval cells. Leucocytosis with a marked shift to the left and the presence of some myelocytes and even myeloblasts is common (Kato and Downey 1933). Examination of erythrocyte resistance shows decrease of the *minimal, and increase of the maximal-resistant* cells, resulting in a broadening of the resistance curve, suggesting *some slight increase in fragility* Heilmeyer (1942) believes this is masked by a greater resistance of the immature forms, especially the thin target cells In moist preparations erythrocytes show tendency to fragmentation (Cooley and Lee, 1925)

In the bone marrow taken at autopsy, Dalla Volta (1935) *Lehndorff (1936), Whipple and Bradford (1936)*, and Schnappoh (1939) found some increase of immature normoblasts, while hæmoglobinized normoblasts were said to be scanty Castle and Minot (1936) reported scanty myelocytes, but numerous megakaryocytes Similar findings have been reported by marrow biopsy Pincherle (1934), Fanconi (*1937*), *Signorelli (1937), Técilazic* (1937) Willi (1937), Panoff (1938) Colarizi and Biddau (1940) Fieschi (1940),

Malamos and Delijannis (1940), Pachioli (1940), Giacomo (1941), Freudenberg and Esser (1942), Diwani (1944) and Fawdry (1944) have recorded hyperplasia of erythropoiesis, which was often enormous, mainly consisting of large immature proerythroblasts (according to Pachioli 70%–88% of marrow cells were early normoblasts). As Pontoni (1937) has pointed out, the marrow even in early cases shows a definite increase of normoblasts, and thus marrow biopsy may contribute substantially to the diagnosis. According to Panoff the marrow reaction is a pathological one because it has lost the faculty of retaining immature normoblasts. Dameshek (1940), on the other hand, describes an "anerythroblastic type" of Cooley's anæmia without normoblasts in the peripheral blood, which he calls "target cell anæmia" on account of the morphological picture. According to Fieschi the mitotic figures more closely resemble those of megaloblasts than of normoblasts. True megaloblasts have not been observed by Kato and Downey (1933), Fieschi (1940), Malamos and Delijannis (1940) and Rohr (1943). We ourselves have so far examined only blood smears, but no marrow material. No megaloblasts were seen in our preparations. Di Guglielmo (1928) as well as Giacomo (1941) reports an increase of prepolykaryocytes and polykaryocytes, but they are probably lobed precursors of the red cells and not precursors of megakaryocytes.

Rohr (1943) has recently recorded a familial hæmolytic anæmia of the Cooley type in adults, which had been described previously by Italian authors (Monigliano-Levi and Barrati, 1937) as constitutional hæmolytic anæmia with elliptocytic hypochromic poikilocytosis. Two patients were examined by sternal puncture by Rohr who found increased erythropoiesis with 150–220 normoblasts per 100 white cells.

Anæmias of the Neo-natal Period and Infancy

Di Guglielmo (1928) classifies all erythroblastoses as follows:—

I (1) Hyperacute erythræmic myelosis of the new born (Rautmann)
(2) Acute erythræmic myelosis (di Guglielmo)
(3) Chronic erythræmic myelosis (Cooley's anæmia)

II (1) Erythroblastosis fœtalis (or hyperacute erythroleukæmia of the new born (v. Gierke)
(2) Erythroleukæmia of infancy (v. Jaksch, Hayem, Luzet)
(3) Chronic erythroleukæmia of adults (di Guglielmo)

This classification, though lucid from the systematic point of view, has the disadvantage that it also comprises leukæmias and other diseases. Lehndorff (1937) states that the simple anæmia of the new born is a monosymptomatic disorder, because, except for pallor no other manifestations become evident, and it undergoes

spontaneous and definite cure The prognosis of anæmia complicated by jaundice is much more serious. Icterus gravis neonatorum has a mortality of about 80%. *Fanconi (1937) has described* milder forms, which he called icterus gravis non letalis, but they are relatively uncommon. The third form of this group is hydrops fœtalis universalis, in which anæmia may be masked by hydrops. The babies often die before anæmia has time to develop and the mortality is almost 100%. Mothers who have borne a child with anæmia of the new born almost invariably bear subsequently children with the same disorder, or with icterus or hydrops indicating some relationship between the syndromes discussed.

The ætiology remained obscure until quite recently. Wintrobe and Shumacker (1936) suggested absence of the anti-pernicious factor. Diamond, Blackfan and Baty (1932), Hellman and Hertig (1938), and Mellinghoff and Randerath (1941), suggested changes *in the placenta, such as* persistence of the layer of Langhans' cells, hyperplastic syncytium, larger villi with hyperplastic and œdematous stroma and other malformations. Langen (1937) suspected toxins of pregnancy, Lehndorff (1937) allergic reactions to placental proteins. Recent observations of English authors, including Haldane (1942), Race, Taylor, Cappell and McFarlane (1943), Gimson (1943), Mollison (1943) and Cappell (1946) have shown that the Rh factor of the erythrocytes, discovered by Landsteiner and Wiener in 1940, is of ætiological importance. When rabbits and guinea-pigs are immunized with the blood of Rhesus monkeys, an agglutinin is formed which agglutinates the erythrocytes of certain persons. Hoare (1943) found on the average 85% of the population were Rh-positive. Among fifty mothers of affected children, Race, *Taylor, Cappell and McFarlane (1943) found only* 6 Rh-positive and 44 Rh-negative, against 7 or 8 Rh-negative to be expected on general population distribution. All 50 children were Rh-positive. Therefore, the assumption is that the Rh-negative mother becomes immunized against the Rh-factor by the Rh-positive child, and the mother produces hæmolytic antibodies against Rh This leads to hæmolytic anæmia and erythroblastosis of the new born In thirty-eight of the fifty mothers an anti-Rh agglutinin was found. It is also important to discover if the fathers are homozygous Rh carriers (RhRh) who are, according to Race and Taylor, four to *five times as dangerous as those* who *are* heterozygous (Rhrh). By transfusing Rh-negative blood it has been [illegible] to keep alive babies with erythroblastosis fœtalis, because

was given, Race and his colleagues saved 25%, [illegible] all the children (sixteen of nineteen). It is, therefore, suggested that Rh-negative blood should be kept in readiness *in obstetric* departments.

The blood picture is characterized by a severe anæmia (R B C 1 million, Hb. 50%) with hypochromia and macrocytosis without anisopoikilocytosis. Erythroblasts are scanty in the simple anæmias, but very numerous in icterus gravis. We have recently investigated an icteric child, in whom 87% of the nucleated cells were normoblasts. Extramedullary hæmopoiesis also is much more pronounced and more persistent in these children.

In the bone marrow, hyperplasia of leucopoiesis and even more of erythropoiesis was observed with an increase of mitoses by Mannheimer (1935), Fanconi (1937), Lehndorff (1937), Vidari (1938), Rohr (1940) and Gilmour (1944) In 3 out of 5 cases examined by sternal puncture, Keel (1943) found very marked normoblastic hyperplasia. There was a normal marrow picture in the other two.

Familial Pernicious-like Anæmia (Fanconi)

This familial pernicious-like anæmia was described by Fanconi in 1927 in three brothers between the ages of five and seven. It is accompanied by leucopenia and thrombocytopenia as well as by endocrine disturbances, such as testicular atrophy. The marrow showed atrophy and fatty degeneration with only small hæmopoietic foci Weil (1938) recorded such cases under the name of aplastic infantile myelosis. In a case reported by Stowers and Dent (1947) some abnormally large megakaryocytes were found in the sternal marrow, but the peripheral blood counts were normal. There was severe osteomalacia, mild diabetes with renal glycosuria, amino-aciduria, hypophosphatæmia and, as a complication, malignant hepatoma of the liver

PSEUDO-LEUKÆMIC ANÆMIA OF CHILDREN (v JAKSCH-HAYEM-LUZET)

This anæmia appears between the seventh and eighteenth month of life. It is characterized by anisocytosis, poikilocytosis, polychromasia, erythroblastosis, leucocytosis with a shift to the left and the presence of myelocytes, and hepatomegaly and splenomegaly It occurs among the children of the poorer classes of the population and is often associated with rickets This condition is not actually a separate disease, but an erythroleukæmic (leuco-erythroblastic) reaction, in which possibly gastric conditions play a part, and secondly also possibly infections Bernard (1943) regards syphilis as the cause, Gittins (1933) believes it is the regenerative phase of a hæmolytic anæmia, Opitz (1931) says it may be a constitutional reaction to nutritional defects In sternal marrow, Teclazic (1937) as well as de Weerdt (1939) found an increase of normoblasts. De

Weerdt found 10·3 basophilic, 116·6 polychromatic and 43·3 pyknotic normoblasts per 100 white cells. Bernard and others found myeloid and normoblastic metaplasia in the spleen and liver.

HYPERFUNCTION OF ERYTHROPOIESIS

ACUTE ERYTHRÆMIC MYELOSIS (DI GUGLIELMO)

This is a rare malignant erythroblastic hyperplasia It was first described by Copelli (1912) and later defined as a separate disease by *di Guglielmo (1923) as acute erythræmia. Moeschlin (1940,* 1947) states that only 5 cases can withstand critical scrutiny: viz, 2 cases reported by di Guglielmo (1928, 1937), but not his first *case studied in 1923, and 1 case each reported by Lazzaro (1933),* Benedetti (1938), and Paradiso and Reitano (1939) The other cases are in fact neonatal anæmias, such as di Guglielmo's case of *a jaundiced baby, who died on the third day of life, and* Teodori's (1938) which only lived for 12 hours. The 2 cases described by Vidari (1938) are also of different origin, one having hydrops and the other icterus Cases of Cooley's anæmia are confused with di Guglielmo's disease even more frequently, owing mainly to the differing terminology used by Italian writers Moeschlin (1940, 1947) regards the following cases in this light Prebil (1931). Lattes (1932), Noto (1933), Paradiso (1933), Miraglia del Giudice (1936), Pouché (1936), Bruera and Picena (1937) and Bocchini (1937) With two exceptions (Miraglia del Giudice, Paradiso) they were children between five and fifteen months Quattrin (1940), who recorded the case of a woman of forty-four, confirmed by autopsy, reviewed the literature and considered 7 other recorded cases as true acute erythræmia in addition to the cases already mentioned Baserga (1938) collected 18 relevant cases, but only a few of these were diagnosed beyond any doubt Similar cases have *been reported by Knoll (1921), Pinkerton (1929) Canale (1930),* Forti (1931), Brugi (1932), Frugoni (1936), Israels (1939) Bruni (1940), Dalous de Brux and Bollinelli (1944) and Fiessinger *Carlotti and Laur (1946), but not all have been confirmed* More recently Bianchi (1939) described the case of a man aged twenty-two but Moeschlin would class this under Erythroleukæmia Duesberg *(1940) has reported 2 cases,* and Chevallier and Ely (1939), Roth (1940), Stodtmeister (1941), Kienle (1942), Scapaticci and Lelli (1942) and Pontoni (1943) have each recorded 1 case The latter was thoroughly investigated by Cajano (1946), and the whole subject has been reviewed by di Guglielmo (1945, 1946)

Owing to the confusion in the interpretation of the cases we must attempt to define Acute Erythræmic Myelosis clinically and hæmatologically, di Guglielmo's criteria are as follows

1. Severe anæmia from the start of the illness.
2. Irregular, usually remitting fever.
3. Splenomegaly.
4. Slight hepatomegaly.
5. Acute course of one to two months, invariably fatal.
6. Erythroblasts in the blood, many atypical and immature.
7. Hyperplasia of erythropoiesis with maturation arrest.
8. Proliferation of the reticulo-endothelial system.

Most authors regard the disease as a systemic disorder, corresponding to leukæmia, but Stodtmeister believes that it is a severe marrow reaction, and Weil and Perlès (1938) a hepato-lienal affection, which may occur in various infections. Pittaluga *et al.* (1940) take up a similar attitude. Dustin (1936, 1937) has recorded a case of polycythæmia, which terminated in acute erythræmic myelosis. The following case was recently studied by one of us (Neumark, 1948):—

Case 19. R. B., bank accountant, aged 39, complained of bleeding from the gums, shortness of breath and pallor for two months and blurring of vision in the right eye for one week. Personal and family history nil relevant. On examination he was pale, well nourished and breathless at rest. Temperature 100.2° F., B.P. 135/70 mm. Hg., petechiæ all over the body. Swollen gums with clotted blood between the teeth. Fauces clear. Offensive breath. Heart with apical systolic murmur. Liver and spleen just palpable. Hess's test positive. Hæmorrhage in the right retina. Diagnosed as acute leukæmia.

Blood. R.B.C. 1.5 millions, Hb. 32% (Haldane) = 4.4 g.%, C.I. 1.0. M.C.D. 7.1μ. W.B.C. 700, polymorphs 24%, lymphocytes 71%, monocytes 5%, nucleated red cells 350 per 100 white cells: proerythroblasts 2%, basophilic erythroblasts 21%, polychromatic erythroblasts 4%, orthochromatic erythroblasts 41%, normoblasts with pyknotic nuclei 32%, 1 erythroblast in mitosis. Anisocytosis, poikilocytosis, polychromasia marked. Slight punctate basophilia, reticulocytes 3%, platelets 18,000. Red cell fragility: 0.375% NaCl complete, 0.4 partial, 0.45 no hæmolysis. Serum bilirubin 0.7 mg./100 ml. W.R. negative, Kahn negative. Prothrombin time 44 sec. Blood culture sterile. Clot retraction 23%.

Sternal Marrow. Cellular marrow: proerythroblasts 6.6%, basophilic erythroblasts 14.8%, polychromatic erythroblasts 11.2%, orthochromatic erythroblasts 46.8%, myeloblasts 1.2%, promyelocytes 8.2%, myelocytes 7.8%, metamyelocytes 0.4%, segmented polymorphs 1%, lymphocytes 0.2%, reticulum cells 0.8%, mitotic figures 1% (all in erythroblasts). Megakaryocytes very scanty (two seen in three films). Platelets very scanty. Myeloid-erythroid ratio 1 : 4.

Other investigations: urine normal. Sputum: pneumococci ++, no Tb. Ascorbic acid saturation test normal. X-ray of chest: marked pulmonary striation, simulating carcinomatous lymphangitis. X-rays of forearm, hand, femur showed no bony abnormality. Progress and treatment: Massive blood transfusions, parenteral liver, iron, penicillin. Intermittent pyrexia 99° F.–103° F. Hæmoglobin rose to 80% after transfusion but fell to 46% within one week. Blood pictures showed persistent neutropenia and lymphopenia. Nucleated red cells fell to 100

per cmm. Splenectomy was considered to be the patient's last chance,

marrow. No significant enlargement of the lymph glands. Spleen 470 g., on section red with the appearance of marrow. Microscopically the liver showed fine fibrosis, and increase of reticulum fibres, most marked in the portal areas; femoral marrow: very cellular, thin bony trabeculæ, the large majority of cells look like basophilic erythroblasts. Occasional foci of eyrthroblastic proliferation. Lymph glands: Malpighian bodies indistinguishable; diffuse erythroblastic metaplasia. Spleen with many erythroblastic cell types.

The striking features of this case were the short duration of the symptoms, the hæmorrhagic diathesis, the progressive and rapid



FIG 112 Abnormal erythroblasts from sternal marrow of a case of acute erythræmic myelosis. (× 1,000)

deterioration, and the intermittent pyrexia. The *blood picture* was characterized by profound anæmia with a colour index about unity, and by neutropenia, and marked erythroblastosis, with many early forms The marrow showed gross hyperplasia of the erythropoietic system with maturation arrest, and hypoplasia of myelopoiesis and thrombocytopoiesis (Fig 112). *Clinically the case* developed like an acute leukæmia Hæmatologically it was a pure, classical case of acute erythræmic myelosis, and fulfilled all di Guglielmo's criteria The diagnosis was confirmed at autopsy

The most important features are the immature erythroblastosis in *the blood and the hyperplasia of the erythropoietic portion* of the marrow with many immature basophilic forms, proerythroblasts

and hæmocytoblasts. Because orthochromatic normoblasts are absent or diminished Baserga (1938) speaks of hiatus erythræmicus completing the analogy to acute leukæmia. Erythropoietic foci are found in the liver and spleen, as well as an increase in reticulum cells. Quattrin (1940), Kienle (1942) and others described hæmohistioblasts and hæmocytoblasts. Kienle believes that the erythroblasts possibly develop directly from the histioblasts, by-passing the stage of the hæmocytoblast. All investigators have found erythroblastic hyperplasia in the bone marrow. In autopsy material, di Guglielmo (1928) and Lazzaro (1933); and in marrow biopsy, di Guglielmo (1938), Benedetti (1938; 85·5% of the marrow cells), Paradiso and Reitano (1939; 88·2% of marrow cells, of which 83% were basophilic), Quattrin (1940; 89% of marrow cells, with 19 hæmocytoblasts, showing a developmental tendency towards the erythroblasts, 25·5 procrythroblasts, 38 basophilic erythroblasts and 6·4 polychromatic erythroblasts), Roth (1940), Duesberg (1940), Nabholz (1942), Kienle (1942), Pontoni (1943) and Cajano (1946; 69·4% basophilic erythroblasts, of which 45·9% were paraforms, 14·2% procrythroblasts, 4·7% polychromatic erythroblasts and 1·1% orthochromatic erythroblasts).

Differential diagnosis from Cooley's anæmia is hardly possible on hæmatological grounds alone, because both diseases are characterized by blood and marrow erythroblastosis. The very well-marked histiocytic reaction and the advanced anaplasia might possibly favour acute erythræmic myelosis. Kienle investigated a child of ten years and described atypical mitoses. He describes amitotic and pseudo-amitotic divisions; in the mitoses the chromosomes were longer and more slender than in normal erythroblasts, and the cytoplasmic rims were broader. He considered these findings indicated precipitate proliferation with maximal inhibition of the maturation curve. The clinical differentiation of Cooley's anæmia is more certain as it occurs in children, has a familial trait, a chronic course and skeletal changes are usual, due to the chronic marrow hyperfunction during childhood.

The myelopoietic reaction is not characteristic. Frequently there is leucocytosis (up to 30,000) with a shift to the left and the presence of myelocytes. In the sternal marrow extreme hypoplasia of granulopoiesis and thrombocytopoiesis is usually seen. As in the acute leukæmias a hæmorrhagic diathesis often occurs.

CHRONIC ERYTHRÆMIC MYELOSIS. (TYPE HEILMEYER–SCHÖNER)

Chronic erythræmias have been described in adults and Heilmeyer and Schöner (1941) consider that they occupy a position analogous to chronic myeloid leukæmia. Benedetti (1938) had previously reported a case of chronic erythræmia and Duesberg (1940) reported

similar cases entitling his paper "anæmias from faulty differentiation of erythroblasts" The case described by Israëls (1939) probably does not belong to this group because of the myeloid reaction present, which places it more in the group of erythroleukæmia, although sternal marrow showed 67 nucleated red and 33 white cells. The disease led to death in four months. This case, as well as one reported by Fieschi (1940), living for four and a half months, may be described more aptly as subacute erythræmic myelosis. Heilmeyer and Schöner's patient was a baker of seventy-five years. A progressive increase of erythroblasts in blood, marrow, liver and spleen was found and the patient died two years after the first observation. In the terminal stage there were more erythroblasts in the blood than white cells (122 : 100); in the marrow there were 3,268 nucleated red cells per 100 white cells; in the spleen 600 and in the liver 756 nucleated red per 100 white cells. Di Guglielmo and Quattrin (1942) reported the case of a girl of twelve and a half years who was equally well investigated and the diagnosis confirmed by autopsy. She had 14,450–16,450 erythroblasts per cmm. in the blood, erythroblastic hyperplasia in the marrow, liver, spleen, kidneys and heart, and bilirubinæmia. She improved temporarily after splenectomy. There was no family history, and no spherocytosis Erythroblastic hyperplasia in the bone marrow is the diagnostic feature and both primitive and senile or old forms take part in this hyperplasia.

ERYTHROLEUKÆMIA (DI GUGLIELMO)

This is a disease with a leukæmic as well as an erythræmic blood picture and proliferation of both systems in the bone marrow The sternal puncture findings are diagnostic Such cases have been described by Penati (1937), under the title of "megaloblastic leukæmia," and by Moeschlin (1940) and Rohr (1940) Moeschlin has expressed the opinion that many cases reported as acute erythræmic myelosis belong to this group, because the histiocyte-like cells could possibly have been interpreted as myeloblasts, particularly so in the case reported by Bianchi (1939) In Penati's case the leucocytes in the blood rose to 83,000 per cmm erythroblasts to 15,900 per cmm among which there were 11 1% megaloblasts in the sternal marrow almost half the cells (47 1%) were erythroblasts 29·6 of them megaloblasts Harvier *et al* (1942) have described a case of acute erythroleukæmia Moeschlin's patient died after an illness of seven months, during which

at first 343 but four months later 31·5 erythroblasts per cells Stabel (1943) has recorded a case of subacute erythroleukæmia with erythroblastæmia (up to 104 nucleated red cells per 100 white

cells), myeloblasts (up to 14), promyelocytes (up to 25) in the blood, and a very cellular marrow with myeloid and erythroblastic hyperplasia. As he also found megaloblast-like cells, he thought that some deficiency shall be considered as a possible causative factor.

LEUCOERYTHROBLASTIC ANÆMIA

While true erythroleukæmia is a rare condition, leucoerythroblastic anæmias have been observed not infrequently. Though in myeloid leukæmia normoblasts are found in the peripheral blood, hypoplasia of erythropoiesis is present in the marrow. Moeschlin (1940) in 74 cases of myeloid leukæmia only once found 30

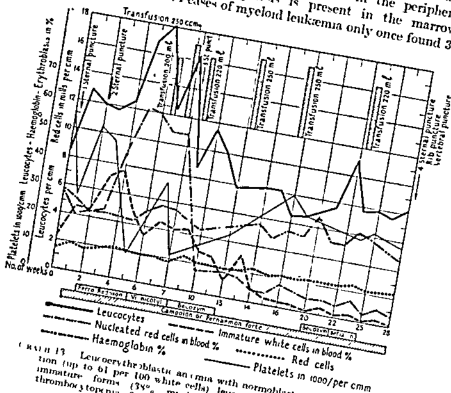


CHART 13. Leucoerythroblastic anæmia with normoblasts in the circulation (up to 61 per 100 white cells) leucocytosis up to 18,100 with immature forms (38% myelocytes and metamyelocytes) and thrombocytopenia of 9,000

normoblasts per 100 white cells in the marrow compared with the usual 1–5. He observed high figures only in temporary remissions, such as in a man of twenty-three years, who had 270 normoblasts per 100 white cells. These are rare and transient marrow reactions. Leucoerythroblastic anæmias may occur in tuberculosis of the spleen, Hodgkin's disease of the spleen and other conditions. Leitner (1945) has recorded a severe leucoerythroblastic anæmia

in a case with carcinomatous metastases in the bones. This case was observed hæmatologically for several months:—

Case 20. S. A., a woman of 45, became ill in October, 1941, *with* lassitude; in January, 1942, she developed pains in the back. She was first diagnosed as sciatica and when spa treatment proved useless the diagnosis of tuberculosis of the spine was made on the grounds of X-ray photographs When admitted to hospital there was no tenderness over the spine on pressure or on tapping [illegible]
definite pain [illegible]

[illegible] spleen was enlarged to the costal margin as judged by percussion.

BLOOD. R.B C. 1 64 millions, Hb 54% = 8·7 g. %, C.I. 1 6; W.B C.

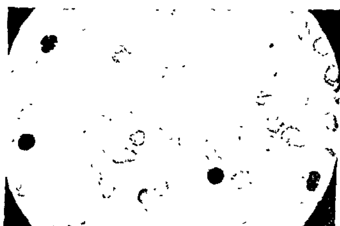


FIG. 113. Blood in leucoerythroblastic anæmia Four normoblasts, two of which are in karyorrhexis (× 650)

8,850; basophil myelocytes 2%, basophils 1·5%, eosinophil myelocytes 0 5%, eosinophils 2%, neutrophil myelocytes 3·5%, metamyelocytes 8·5%, stab forms 17%, segmented polymorphs 27·5%, lymphocytes 23 5%, monocytes 10 5%, reticulocytes 7·5%, aniso-poikilocytosis, normoblasts 22 5, early normoblasts 4·5, procrythroblasts 1, megaloblast like cells 0 5 per 100 white cells Serum iron 68γ%, bilirubin direct negative, indirect 0 25 mg %, non-protein-nitrogen 21 5 mg.%, total serum protein 5 83 g.%, albumen 2 8 g %, globulin 3 03 g % fibrinogen 0 17 g %, Takata-Ara reaction positive

STERNAL MARROW Early basophilic normoblasts 0 3, normoblasts 17 6 per 100 white cells, myeloblasts 0%, promyelocytes 2%, semi mature myelocytes 2·3%, mature myelocytes 13 6%, metamyelocytes 9%, stab forms 10·3%, segmented polymorphs 36 6%, eosinophil myelocytes 0 3%, eosinophil [illegible] lymphocytes 16%, monocytes 3[illegible] lymphoid reticulum cells 0 3[illegible] puncture material small gro[illegible] whole the marrow was rather poorly cellular and showed hypoplasia of erythropoiesis and leucopoiesis Subsequent sternal punctures gave similar results.

The prognosis was unfavourable. In spite of blood transfusions, liver, iron, Becozym (a Vitamin B product), lactoflavin and nicotinamide, etc., the anæmia did not improve, nor did the clinical state. In post-mortem punctures of the sternum, ribs and vertebræ we found the same, but rather bigger groups of tumour cells The leucoerythroblastic reaction increased greatly for a short while, as shown in Graph 13.

In short—a woman with carcinomatous deposits in the bones showed leucoerythroblastic anæmia in which the nucleated cells totalled 18,100 The figures for erythroblasts reached 61 per 100 white cells. At the beginning primitive erythroblasts were present (on September 23rd, six proerythroblasts and twelve early normoblasts), but later the more mature normoblasts with bizarre karyorrhexis and punctate basophilia predominated (Fig 113) In the white series, the shift to the left was marked and myeloblasts were present up to 1·5%, the maximum number of myeloblasts, promyelocytes, myelocytes and metamyelocytes was 38% (Fig 114) It was interesting to note the leukæmoid reaction in all three granulocytic systems, analogous to true myeloid leukæmia



Fig 114 Two myelocytes in peripheral blood in leucoerythroblastic anæmia (× 1,400)

The number of platelets was always low and varied between 9,000 and 54,000, but a hæmorrhagic diathesis did not appear Towards the end of the illness the leucoerythroblastic reaction retrogressed, but it could be demonstrated up to the time of death The analogy with true erythroleukæmia was striking. Sternal punctures showing a hypoplastic, poorly cellular marrow and a reduction of erythroblasts, hypoplasia of the granulocytopoietic portion of the marrow and groups of tumour cells (see Chapter XIII) established the diagnosis Similar cases have been recorded by Mach and Klages (1930) and Vaughan (1936).

POLYCYTHÆMIA VERA (VAQUEZ-OSLER) AND ERYTHROCYTOSIS

Like Schulten (1939) we feel that it would be of great importance if we could distinguish between erythrocytosis and polycythæmia vera by means of sternal puncture An agreement on nomenclature to avoid confusion would also be helpful Many French and American authors call polycythæmia "erythræmia," others call it "polyglobuly" Weil *et al* (1939) talk of "simple polyglobuly" with hyperplasia of all three systems, which would correspond to poly-

cythæmia vera, and of other types of polyglobuly, *with a relative* increase of the red cells. The term polycythæmia should be restricted to a disorder, in which erythrocytes, leucocytes and *platelets* are increased The distinction of a type Vaquez with splenomegaly, and a type Gaisböck with hypertension, appears *unnecessary and* often impossible, because hypertension and splenomegaly may be present in one and the same patient.

Although the symptomatology with lassitude, *headaches*, noises in the ear, giddiness, etc., is quite clear, *there* is *much* discussion about the ætiology of the disease. Morris *et al.* (1932), Hitzenberger (1934) and Briggs and Oerting (1935) suggest that polycythæmia is the exact opposite to pernicious anæmia and depends on an overproduction of Castle's *factor*. Baráth and Fulöp (1935) have attempted unconvincingly to prove this theory with Singer's reticulocyte *reaction in rats*. *Improvement* has been recorded by Hitzenberger (1934) after resection of the pyloric region, which is presumed to be the site of production of Castle's factor, and by Andersen, Geill and Samuelsen (1938) after irradiation of the pyloric region. Stenstrom, Hallock and Watson (1940) have been unable to confirm these findings. Franke (1943) transfused 1 litre of blood from polycythæmic patients to patients with pernicious anæmia without any success and therefore does not accept Hitzenberger's theory Töttermann (1942) failed to induce polycythæmia by the administration of liver extracts to healthy subjects Stöger (1943) administered a mixture of meat and gastric juice from polycythæmic patients to patients with pernicious anæmia and observed higher figures for reticulocytes than when he gave normal gastric juice He, therefore, firmly believes that there *is* an increase in the intrinsic factor in polycythæmia

Tischendorf and Herzog (1940) draw attention to the parallel features between leukæmia and polycythæmia, such as splenomegaly, *marrow hyperplasia and possibly extramedullary* hæmopoiesis, and the incurability But, of course, the progress of polycythæmia is more chronic and more benign Moeschlin and Rohr (1939) also placed polycythæmia in a position parallel to leukæmia This opinion is favoured by the success of the treatment of polycythæmia vera with radioactive phosphorus (Erf and Jones 1943, Hall *et al.*, 1945, Warren, 1945, Erf, 1946; and others) Erf produced satisfactory clinical and hæmatological improvement in *17 of 25 patients* and remissions in 3 patients, the dosage given was 5–10 Millicuries (in 10–20 ml solution), of a preparation with a half *life* of 14·3 days Bakalos and Thaddea (1943) consider polycythæmia to be a reactive systemic hyperplasia, *which* profoundly differs *from* leukæmia Naegeli (1931) suspected a disturbance of correlation as the causal *agent just as in leukæmia*, while Dedichen (1941) and Nordenson (1946) term the disease erythroblastosis of the adult

Dittmar (1939) as well as Symanski (1935) observed polycythæmia in carbon-monoxide poisoning Dittmar believes this is due to damage to the mid-brain by carbon monoxide. Schulhof and Matthies (1929) and Castex (1931) actually produced polycythæmia experimentally by damage to the mid-brain. Naegeli thought that the increased basal metabolic rate was due to hyperactivity of the marrow, but damage to the metabolic centres in the mid-brain is possible. Coppo and Serafini (1943) believe the cause is more likely to be found in the pituitary or the mid-brain, rather than in a hypersecretion of the gastric juice Loeper, Varay and Chassagne (1941) believe that polycythæmia develops as a compensatory measure to increased endogenous CO_2 tension of the blood. Mondon and André (1941) observed polycythæmia after benzol intoxication Ziegler's (1924) theory of a speed-up of cell maturation and of dispatch into the blood stream, Luce's (1903) theory of an abnormal toughness of the erythrocytes, and Burger and Beumer (1913) and Lommel's (1908) theory of the existence of an inferior type of hæmoglobin have not been substantiated

A definite increase of erythroblasts in bone marrow has been observed by Askanazy (1927) and Naegeli (1931) in post-mortem material and by Weiner and Kaznelson (1926), Zadek (1927), Dameshek (1933) and others by biopsy Révol (1938), who found 61% erythroblasts, states that so high a figure of erythroblasts excludes symptomatic erythrocytosis Weiner and Kaznelson (1926), Nordenson (1935), Piccena (1937), Sansone (1937), Klima (1938), Henning and Keilhack (1939), Markoff (1939), Morrison and Samwick (1939), Rohr (1940), Thadden and Bakalos (1940), Dedichen (1941), Leitner (1941), Heilmeyer (1942) observed hyperplasia of the white series and of megakaryocytes also, but Zadek (1927) did not find any increase of the megakaryocytes and Tischendorf and Herzog (1940) frequently did not find an increase of platelets Kienle (1943) believes that the increase of megakaryocytes is not of importance Nolli and Benario (1936) reported figures for erythroblasts and proerythroblasts of 32%–68%, and also immaturity of the myeloid series Roversi and Tanturri (1935) found over 45.89% of normoblasts and a stimulation type of maturation curve for the myeloid series Mallarmé (1937) reported an increase of proerythroblasts with hyperplasia of megakaryocytes Whitby and Britton (1946) found a great increase of the erythroblastic and leucoblastic tissue as well as of the megakaryocytes The increase was found among all types of normoblasts, myelocytes and even myeloblasts, proerythroblasts, however, were not affected According to Schilling (1933), Fieschi (1940) and others the marrow is hyperplastic, but Fieschi found a normal maturation curve of the myeloid series and does not believe that sternal marrow puncture is of much value in diagnosis, because the volume of marrow is increased, and this may be the cause of an apparent increase of cells

In our opinion, however, this does not diminish the diagnostic importance of sternal puncture. Vischer (1938) observed normoblastic hyperplasia in most but not all of his cases, while Klima (1938) in his 10 cases found the number of the normoblasts only once to be greater than 50%. De Weerdt (1939) in 3 cases failed to observe an increase of normoblasts, or hyperplasia of the granulocytes, but he did note an increase of megakaryocytes in one case. Westenhöfer (1907), Löw and Popper (1908) also frequently failed to find a proportional increase of normoblasts. Nordenson, Fieschi and Kienle have reported an increase of mitotic figures. Some of the discrepancies in findings may be due to the fact that if all three systems are hyperplastic the relative proportions of each may be unchanged so that unless a total nucleated cell count be carried out, hyperplasia may not be recognized. In most of our cases we have found definite hyperplasia of all three systems.

Case 21. F. F., a chauffeur of 42 years, had had measles, pneumonia and influenza. In February, 1938, he became ill with tuberculosis of the epididymis and kidneys. Epididymectomy and left nephrectomy were performed. The patient was corpulent, 95 4 kg., 173 cm tall, his face showed fine red vessels. His father was said to have been even more corpulent and more red-faced. Blood pressure 170/115 mm Hg

BLOOD. R.B.C. 6·24 millions, Hb 124% = 19 9 g.%; W.B.C. 10,900; basophils 1%, eosinophils 2%, stab forms 1%, segmented polymorphs 56%, lymphocytes 32 5%, monocytes 7·5%; platelets 635,000 (Fonio's method). Sedimentation rate (Westergren) 2–6 mm. (1 and 2 hr.).

[illegible] cytes 11%, stab forms 6 6%, segmented polymorphs [illegible] myelocytes 4%, eosinophil metamyelocytes 3%, eosinophils 2 6%, lymphocytes 6%, monocytes 0 6%, megakaryoblasts 0 3%, megakaryocytes 4%, plasmoblasts 0 3%, plasma cells 1%, lymphoid and phagocytic reticulum cells 0·3%.

The puncture material was very cellular, erythroblasts numbered more than 60 per 100 white cells. Myelocytes and promyelocytes were increased, as well as the number of marrow giant cells In the peripheral blood, erythrocytes, leucocytes and platelets were correspondingly increased

Other cases are not always as typical as Case 21, but differentiation from symptomatic erythrocytosis has always been possible Weil *et al* (1939) record atypical findings Their report of 12% megaloblasts is probably due to a difference in nomenclature Hyperplasia of granulocytes may give rise to confusion with leukæmia, according to Klima, especially when the total figure for white cells in the blood is very high (up to 100,000) and when there is a shift to the left Heilmeyer (1942) in 1 case with myelocytic shift to the left could not find any erythroblasts in the marrow *These exceptional cases do not impair the diagnostic importance*

of sternal puncture. In our 8 cases we have not met any atypical ones.

In contradistinction to polycythæmia we found in symptomatic erythrocytosis a rather poorly cellular marrow with a relative increase of erythroblasts, but with no increase of myelocytes or megakaryocytes.

Case 22. T. E., a girl of 10 with congenital pulmonary stenosis and septal defect. When examined she was cyanosed, had dyspnœa, pain in the precordium, clubbing of the fingers. The left border of cardiac dullness was one-half finger's breadth outside the mid-clavicular line, right border 3 cm. from the middle of the sternum. Soft systolic murmur in the 4th interspace just beside the sternum. Blood pressure 85/60 mm Hg. X-ray of chest: slight cardiac enlargement to the left, less to the right. Electrocardiogram: right axis deviation, some myocardial damage. Fundus oculi: pronounced hyperæmia, engorged veins, edges of disc blurred, flame-shaped hæmorrhages. Liver: three fingers below costal margin, spleen not definitely palpable. She had recurrent attacks of loss of consciousness and died with intensely painful dyspnœa. The autopsy confirmed the diagnosis.

BLOOD. R.B.C. 11.3 millions, Hb. 150% = 22 g.%, W.B.C. 4,400; eosinophils 5%, segmented polymorphs 54%, lymphocytes 31.5%, monocytes 9.5%, platelets 207,000 (Fonio's method). Bleeding time prolonged (eight minutes with Duke's method), clotting time also prolonged (twenty-one minutes with Bürker's method).

Owing to the tremendous erythrocytosis, the venous blood, when allowed to stand, did not produce any plasma, even on centrifugation. When examined with a capillary microscope the capillaries of the nail folds were found to be elongated and dilated.

STERNAL MARROW. Poorly cellular marrow, proerythroblasts 5.25, early normoblasts 6.25, normoblasts 161.75 per 100 white cells, myeloblasts 3.25%, promyelocytes 2.25%, semimature myelocytes 2.75%, mature myelocytes 11.25%, metamyelocytes 12.5%, stab forms 17.25%, segmented polymorphs 25.5%, eosinophil myelocytes 2.75%, eosinophil metamyelocytes 3.25%, eosinophils 2.75%, lymphocytes 9.5%, monocytes 1.75%, plasma cells 1.75%, endothelial cells 1.75%, megakaryocytes 0.75%, reticulum cells 1%. Numerous mitoses in erythroblasts.



FIG. 115. Sternal marrow with increase of normoblasts in erythrocytosis. A proerythroblast and some normoblasts. (× 500)

In this case the CO_2/O_2 ratio in venous blood was 13.86 : 8.56 vol.%, and erythrocytosis was due to an attempt by the body to compensate for the extreme deficiency of oxygen. The number of leucocytes and of platelets was diminished. In the sternal marrow there was a relative hyperplasia of normoblasts (Fig. 115) in a rather acellular marrow, and hypoplasia of granulocytopoiesis and megakaryocytopoiesis.

The normoblastic hyperplasia was mainly made up by polychromatic and orthochromatic normoblasts, but the basophilic ones were also increased. Pachioli (1942) confirmed our findings in a series of small children with congenital cardiac defects. When

erythrocytosis is less severe, normoblastic hyperplasia is also less pronounced, as Otto (1935) has shown in patients with heart disease. In cardiac patients with moderate erythrocytosis we have not always observed hyperplasia of erythropoiesis, though in the majority of cases slight degrees of normoblastic hyperplasia could be detected. The following case of erythrocytosis in a case of Boeck's sarcoidosis has been described in greater detail elsewhere (Leitner, 1942):—

Case 23. W. R., aged 54, had had pneumonia with empyema at the age of 26, treated by rib resection. For the last six years he had had cough with sputum, lassitude and shortness of breath. When examined he was 166 cm. tall and 67·5 kg. in weight. Cervical lymph glands the size of peas. X-ray of chest: small speckled, miliary shadows on both sides. Thickening of pleura on the right. Tuberculin test negative, 1 in 5,000, sputum, no tubercle bacilli; cultures from sputum, urine, fæces, blood and sternal marrow: negative.

BLOOD. R.B.C. 6·65 millions, Hb. 120% = 19·2 g %. W.B.C. 6,300, *eosinophils 8%, stab forms 1%, segmented polymorphs 48%*, lymphocytes 37%, monocytes 7%. Platelets 181,000; sedimentation rate (Westergren) 1–5 mm (1 and 2 hr.).

STERNAL MARROW. Pr[illegible] normoblasts 48·3 per 100 white [illegible]

[illegible]

cells 4·3%, phagocytic re[illegible]

fat cells 0·6%, unidentified cells 0·3%

There was moderate but definite hyperplasia of erythropoiesis while granulocytopoiesis and megakaryocytopoiesis were not involved. We believe that the erythrocytosis was due to Boeck's sarcoidosis especially of the spleen, but the cardiac lesion could not be excluded as a causal factor owing to the overloading of the *right side of the heart.* Two years after discharge this patient returned for follow-up. Electrocardiogram then showed evidence of disease of the right coronary artery. Erythrocytosis persisted though to a lesser degree (R.B.C. 6·4 millions, Hb. 116% = 18·6 g %).

Summary. Sternal marrow biopsy has contributed little to the understanding of the ætiology of polycythæmia. Moeschlin and Rohr and Tischendorf and Herzog's conception of the neoplastic nature of the disease is apparently based on the clinical course. It cannot be supported by sternal marrow examination, which failed to show abnormal cells, but showed mostly mature erythroblastic marrow, normally developed myeloid cells and megakaryocytes. Apitz (1940) has emphasized that the final product of this hyperplasia is a mature erythrocyte, which is an important point against the neoplastic theory. The same applies, we believe, to the leucocytes and platelets. *The disease is a panmyelohyperplasia*, which is slowly progressive, and whose ætiology is still uncertain. There is

no proof of its development from central nervous causes. Sternal puncture is a valuable aid to diagnosis and in doubtful cases ensures differentiation from erythrocytosis. Polycythæmia vera is characterised by hyperplasia of all three systems, but symptomatic erythrocytosis is merely a hyperplasia of the red cell system.

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CHAPTER IX

DISORDERS OF LEUCOPOIESIS

THE LEUKÆMIAS: CLASSIFICATION AND ÆTIOLOGY

MARROW biopsies have renewed interest in the quest of the ætiology of the leukæmias. The following four princi theories will be discussed in this section.—

(1) Leukæmia as a systemic disease, following a disturbance correlation.

(2) The infective theory.

(3) Leukæmia as a reactionary hæmopathy.

(4) The neoplastic theory.

The theory of Naegeli (1931), maintaining that the leukæm should be regarded as systemic disorders following upon dysfu tion of the endocrine glands, cannot be supported as close stu has not yet disclosed any relationship to hormonal disturbanc The same applies to the conception of Ferrata (1921) and Fies (1936), who considered leukæmia to be a systemic disorder due t disturbance of correlation between the different cell systems.

The infective theory of the ætiology of leukæmia also finds support in recent work. This theory was previously expounded Ebstein (1887) Decastello (1925), Sternberg (1926), Bettoni (192 Arneth (1930), Krehl (1933), and others, and has recently be revived It was based not only on the likeness of the clini progress of the acute leukæmias and of infections observed Muller (1931), Morawitz (1931) and others, but also on the similar of the blood and marrow findings, pointed out by Ferrata (192 Hoff (1934), Henning (1936), Voit and Landes (1938) and Nopor (1938) Positive blood cultures, reported by Sternberg (190 Hulse (1931) and others have been rightly considered by Naeg due to secondary infections We have ourselves reported a case acute lymphatic leukæmia confirmed by autopsy, in which t blood culture taken during life grew Streptococcus viridans infections did play a part in the ætiology of the leukæmias we shou expect to find positive bacteriological cultures in chronic leukæmi also A positive blood culture, however, only occurs with a frequency in the terminal stages, when the protective powers of t body are already overwhelmed We have carried out many blo and marrow cultures in various leukæmias, without ever obtaini a positive result (Table 21) Hulse is the only one who has succeed in producing leukæmia in guinea-pigs with a strain of streptococ and his results have not been confirmed by other workers. In t section on leukæmoid reactions we shall discuss the reasons why

refuse to accept the view that infectious disease, for example tuberculosis, may damage the hæmopoietic organs in such a way that leukæmia develops. The only evidence in favour of the infective theory is to be found in the leukæmia of animals. In mice, lymphatic leukæmia has been transmitted by injections of blood, but this has never occurred in human patients. Fowl leukæmia is transmissible in series by cell-free filtrates of the leukæmic tissue. Engelbreth-Holm (1941) has reviewed these animal leukæmias.

It is more difficult to bring arguments against the theory that acute leukæmia is a blood disease reaction in which infections, chemicals and pharmaceutical drugs and other factors are of ætiological importance. Stodtmeister (1940) speaks of "myeloblastic reaction" whose clinical picture corresponds, it is alleged, to myeloblastic leukæmia. Hoff (1942) believes that agranulocytosis, aplastic anæmia, panmyelophthisic anæmia and acute leukæmia are merely different phases of one and the same disease, which he called "myeloid insufficiency." He is, however, prepared to retain the various terms, as they correspond to the definite clinical pictures caused by the disease. Other authors such as Voit and Landes (1938) hold similar views. Hoff completely separates these syndromes from chronic leukæmia, which he considers, however, is able to prepare the ground for myeloid insufficiency so that the leukæmia may turn into agranulocytosis or panmyelophthisic anæmia. Hoff apparently recognizes acute phases of leukæmia, but he explains the occurrence of transition from acute to chronic leukæmia by stating that such examinations have been made in an acute phase of an already existing chronic leukæmia. According to Palmén (1943) panmyelopathy may occur in leukæmias both as a prodromal and as a terminal stage. Rohr (1936) is certainly right when he states that the transformation of leukæmia into agranulocytosis is very rare, and is not an integral part of the disease Hyperplastic reactions in agranulocytosis have been observed, and Hoff regards these as attempts at spontaneous cure. They are, however, not very common, and do not belong to the essential picture of agranulocytosis

We certainly agree with Hoff, that the classification of disorders of the blood must be based on clinical and hæmatological findings, but because leukæmia and agranulocytosis or panmyelopathy may produce similar clinical and hæmatological pictures, we are not justified in presuming their identity Stodtmeister and Buchmann (1941) have stressed the contradictions which result when one attempts to classify these diseases and syndromes together However, because they have observed cases in which a myeloblastic reaction in the blood developed later into acute myeloid leukæmia, they presume, as also does Glanzmann (1942), that under the influence of various stimuli a myeloblastic reaction can be transformed into an acute myeloid leukæmia In our experience myeloblastic leukæ-

moid reactions are rare. We believe with Moeschlin and Rohr (1939) that the nature of the two diseases is fundamentally different. In the leukæmias the pathological process is not a gradually progressive or a sudden maturation arrest at the stage of the myeloblast, but rather one of new overgrowth of leukæmic cells. Certainly one cannot alter the diagnosis according to the result of the disease, *i.e.*, presume a "reaction" in cases of cure, and leukæmia in cases with an unfavourable end. Re-interpretations in the light of further evidence also do not enhance the science of clinical medicine, such as happened in a case which was published by Gloor (1930) as myeloblastic leukæmia and subsequently interpreted by Moeschlin and Rohr (1943) as a leukæmoid reaction or aleukia. Unless conclusive morbid anatomical evidence is obtained or clinical observation has continued for a long time (for example the demonstration of myeloblastic marrow by sternal puncture prior to the manifestation of leukæmia), it is much better to avoid drawing far-reaching conclusions to fit in with a preconceived theory. Hæmatological findings are, however, usually sufficient to make a definite diagnosis. Classification into various categories according to the clinical nature of the diseases should not be omitted because of a few difficult borderline cases. Thaddea and Bakalos interpret as leukæmia, certain doubtful cases of agranulocytosis in which pathological monocytes were found in the peripheral blood, and where there was a hæmorrhagic diathesis. We have seen such cases of panmyelopathy confirmed by autopsy (Case 41, p. 60) Heilmeyer's (1942) conclusions are more acceptable. He believes that paramyeloblastic leukæmia is a separate entity, though leukæmias and aleukias may be very similar. Though we (Leitner, 1939) have seen paramyeloblasts and especially parapromyelocytes in panmyelopathy, and in leukæmoid reactions, they are very rare in these disorders, and may be taken to point primarily to leukæmia. Paramyeloblasts and parapromyelocytes are myeloblasts and promyelocytes with abnormal nuclear characteristics, *e g*, indentation and lobation of the nucleus. They may thus be mistaken for monocytic cells.

The fourth theory of the ætiology of leukæmia, which has been much discussed during the last few years, is that it constitutes a neoplastic process This theory was expounded by Banti (1904) and Ribbert (1907), and Sternberg (1915) applied it to the leucosarcomata Hirschfeld (1925), Askanazy (1940), Apitz (1940), Rohr (1943) and others have supported this view. The points against the neoplastic theory are :—

Absence of destructive growth
Absence of localized tumour formation
Absence of metastases

These arguments have been countered by Apitz (1937), Rössle (1939) Askanazy (1940) and Wienbeck (1942) on morbid anatomical

grounds and by *Moeschlin and Rohr (1939), Tischendorf (1942)* and *others on clinical grounds.*

We believe that the most important arguments in favour of the neoplastic theory are based on chloroma and multiple myeloma *which show local tumour development* and destruction of bones together with leukæmia in the peripheral blood.

Chahovitsch and Ignjatschev (1940) point out the destructive processes in liver and kidneys in myeloid and lymphatic leukæmia. Rohr *(1943)* has described marrow biopsies which suggest the first beginning of myeloid leukæmia, but in our opinion the evidence is not convincing. The third argument, i.e., absence of formation of metastases cannot be accepted because the extra-medullary metaplasia may be interpreted as a metastatic process, though *Ziegler (1906) and Helly (1927) used the words "colonization"* or "innidation." Rohr does not consider that the absence of the capacity to metastasize is a valid argument against a neoplastic origin. The arguments against this theory collapse if we assume with Apitz (1937), Hansen (1941) and others, that there are no basic differences between lymphatic leukæmia and lymphosarcoma and that transitional states occur. "Metastatic" lymphatic leukæmia has moreover been observed in lymphosarcoma. The simultaneous occurrence of tumour and leukæmia has also been reported by Gittins and Hawksley (1933) v. Bonsdorff (1937), Ahlström (1938), Weil, Perlès and Fourest (1939), Forconi and Carere-Comes (1940), and others, and Apitz regarded the tumour formation as part of the leukæmic process. In our own experience differentiation on clinical grounds may not always be very easy, because leukæmoid reactions do occur in carcinoma. At autopsy, too, it must be remembered that a growth may alter the picture of leukæmia. Wienbeck (1942) maintains that the retention of the normal anatomical pattern of the marrow cavity would point against leukæmia.

The local tumour formation depends largely on the sort of cells affected. Reticulum cells, being essentially fixed cells, tend to tumour formation, but metastasize rarely and rarely become disseminated, while the mobile myeloid cells seldom permit the recognition of a primary tumour. Lymphatic cells are halfway between reticulum and myeloid forms, and all three phases—primary tumour, mestastases and general dissemination—are equally common. *According to Rohr,* generalized forms may be aleukæmic as long as they are confined to the glands, and only become leukæmic after the involvement of spleen and liver. Rohr credits the spleen with a principal part in the development of myeloid leukæmia because he has observed myeloblasts in a vessel of otherwise normal marrow and in the peripheral blood. We believe that the primary importance of the marrow in the development of leukæmia is unassailable.

Thaddea (1943) brings forward further arguments against the

neoplastic theory, namely the absence of autonomic growth, the absence of monocentric development or local tumour formation, and its peculiarly systemic character. These arguments have been contradicted mainly by observations on multiple myeloma and chloroma and on the corresponding plasma cell leukæmia and chloroleukæmia.

The following reasons have been put forward in favour of the neoplastic theory :—

Clinical and Hæmatological Observations

Moeschlin and Rohr (1939) rely on cellular abnormalities :—

(*a*) Disturbance in the nucleus-cytoplasm relationship.

(*b*) Abnormal size of nucleoli (Zadek, 1933 ; McCarty, 1936)

(*c*) Individual cell character.

(*d*) Disturbances of maturational tendency and discrepancy between nuclear and cytoplasmic maturation.

(*e*) Decreased number of chromosomes.

Tischendorf (1937) on the basis of his observations of 16 cases of myeloblastic leukæmia believes that nuclear anomalies (micromyeloblast and paramyeloblast formation) and lack of correlation of nuclear-cytoplasmic maturation are weighty arguments in favour of the neoplastic theory Stodtmeister and Buchmann (1941) expound similar views. With Moeschlin and Rohr (1939) we believe that transformation of the profoundly pathological cells of acute myeloid leukæmia into normal leucocytes does not exist, nor are there any transitions between the two groups ; the development of acute myeloid leukæmia, therefore, is not to be represented as a progressive maturation arrest, involving ever more primitive cells, but as a crowding out of the cells of normal leucopoiesis by pathological myeloblasts In chronic leukæmias and especially in the lymphatic form with morphologically normal lymphocytes, on the other hand, a slowly increasing shift to the left is more probable

Andres and Shiwago (1933) also discuss further points in favour of the neoplastic nature of leukæmia, viz., observations of abnormal mitotic figures (also reported by Hittmair, 1928), such as alterations of the structure of the chromosomes, their partial amalgamation or their dissolution , the occurrence of multipolar karyokinesis , the dispersion of chromosomes and changes in the number of mitoses Fieschi (1936), however, considers that these phenomena do not constitute proof for this theory as they also occur in normal tissue (Caffier, 1928) We have not seen any such deviations from the normal in normal bone marrow but we have, however, described a particular anomaly of cell division, which occurs only in severe myelopathies and leukæmias, e g , the dissociation of nuclear and cytoplasmic division leading to multinuclear cell formation

Infections can only be considered as activating stimuli in the development of leukæmia, as *marrow biopsies even prior to infection* reveal a myeloblastic leukæmic marrow. In our opinion leukæmias develop metaplasia in the extramedullary *part of the reticuloendothelial* system, as well as colonization from *the marrow and formation* of actual metastases. *Colonization cannot actually be identified* with the metastatic process, because the latter has a further malignant growth of its own. The unfavourable *course of infections in* leukæmia is due to the low functional power of the reticuloendothelial system, which normally carries the burden of defence.

The Factor of Heredity

Petri (1933) collected 33 cases from families from the literature, and other cases are described by Morawitz (1936), Curschmann (1936) Wullenwerber (1937), Ardashnikov (1937), Gottlebe (1938), Laub (1939), Heilmeyer (1942) and Rohr (1945). The majority were cases of lymphatic leukæmia. Since the factor of heredity is not yet clear as far as malignant tumours is concerned, the quoted cases carry little weight as an argument for the neoplastic theory.

Spontaneous Animal Leukæmias

These observations are more important. In spontaneous leukæmia in mice all stages of transitions between local tumour formation and overt leukæmia have been described Furth (1933), Engelbreth-Holm (1941) and others were able to produce leukæmia as well as sarcoma in fowls by filtrable agents Storti and Brotto *(1940) believe that the virus of fowl leukæmia and that of fowl* sarcoma are related or possibly identical. Leukæmias in mice may be *transplanted by cells and intravenous injections of* the cells usually lead to leukæmia, while subcutaneous injections cause local *tumour formation (Richter and McDowell, 1930;* Furth, Seibold and Rathbone, 1933). Storti (1937), Bisceglie (1937) and Storti *and Brotto (1940)* consider that mouse leukæmias have the character of tumours, and the metastases in the liver and spleen develop by the transfer of pathological marrow cells.

Experimental Animal Leukæmias

This provides further support for the neoplastic theory. In mice, leukæmias or leukæmoid pictures have *been produced by* benzpyrene and *methylcholanthrene (Furth* and Furth, 1936, *Storti and Storti, 1937; Gaetani and Lanza, 1937, Morton and* Mider, 1938); by indol (Büngeler, 1932), by benzol (Lignac, *1932),* and *coal tar (Bernard, 1936)* Krebs, Rask Nielsen and Wagner (1930) and Furth and Furth (1936) could greatly increase the

morbidity from leukæmia in mice by irradiation. Leukæmias in man from benzol have been reported by Delore and Borgomano (1928), Weil (1932), Falconer (1933), Sabrazès, Bideau and Glaunès (1937), Penati and Vigliani (1938) and Paniagua (1942). Nielsen (1932) collected 16 cases of leukæmias following X-rays and cases have also been described by Engelbreth-Holm (1937), Weil (1937), Laubry and Marchal (1932) and Weitz (1938).

Studies of Metabolism

Observations on the metabolism of leukæmic cells by Warburg's apparatus failed to produce any conclusive results Kempner (1939) found that myeloblasts and lymphoblasts had a purely oxidational metabolism without the formation of lactic acid similar to other primitive embryonic cells. Contradictory findings were reported by Daland and Isaacs (1927), Fujita (1928) and Bungeler (1932).

Summary. Much material has been collected in favour of the neoplastic nature of the leukæmias, especially in spontaneous and experimental animal leukæmias. Hæmatological investigations by sternal marrow biopsy have led to results which can be interpreted in the same direction. Considering all the work quoted, especially that on multiple myeloma and chloroma, and plasma cell and chloro-leukæmia, we are inclined to regard the leukæmias as a type of neoplasm. They do, however, merit a special position, owing to their peculiarities, and the very variable picture of acute and chronic leukæmias.

The classification of the leukæmias put forward by Rössle (1939) has been widely accepted, and has been modified by various authors, principally by Rohr. It is reproduced here (Table 9) with minor alterations. It contains many hypothetical entities, such as retothelioma and myelocytoma, but expresses the relationship very well.

TABLE 9

Tissue of origin	Reticulum		Plasma cells	Lymphoid tissue		Myeloid tissue	
	Primitive	Mature		Primitive	Mature	Primitive	Mature
Primary tumour	Reticulo sarcoma	Reto thelioma	Myeloma (plasmocytoma)	Lymphosarcoma	Lymphoma	Myelosarcoma	Myelocytoma (?)
Systemic affection	Retothelsarcomatosis	Reticulosis	Multiple myeloma	Lymphosarcomatosis	Aleukæmic lymphadenosis	Aleukæmic myeloblastosis	Aleukæmic chronic myelosis
Leukæmia	Monocytic leukæmia		Plasma cell leukæmia	Acute lymphatic leukæmia	Chronic lymphatic leukæmia	Acute myeloid leukæmia	Chronic myeloid leukæmia

CHRONIC MYELOID LEUKÆMIA

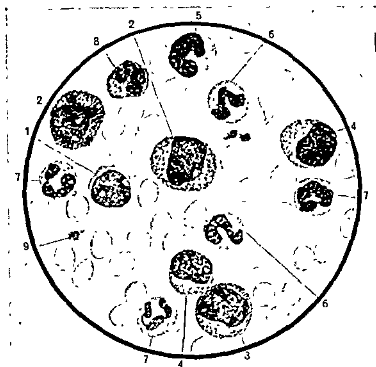
In myeloid leukæmia sternal marrow biopsy produces a picture very similar to that found in the peripheral blood, and sternal puncture, therefore, is not of diagnostic importance. The blood picture with a high leucocyte count (100,000–300,000 or more), and with a marked shift to the left of the myeloid cells with myelocytes up to 30%, usually settles the diagnosis. In the initial stages, when the blood picture is not fully developed, sternal puncture fails to help in the diagnosis, because the number of myeloblasts is not appreciably increased, and differentiation from reactions to infections is therefore not easy (Barta, 1932) The dilution effect of admixture of blood must also be remembered, because leukæmic marrow is thick and diffluent, and the examination of small pieces of marrow presents some difficulties (Viola, 1927; Escudero and Varela, 1928 Introzzi, 1935; Leitner, 1941).

Klima and Seyfried (1937) point out a number of characteristic points of morphology which are typical for leukæmia, and Naegeli (1931) first described the uneven maturation of the cytoplasm. Indentation of the nucleus is evidence of the maturity of the normal myelocyte, but immaturity is shown by a perinuclear zone of basophilic cytoplasm with dark coarse granulation, which becomes lost towards the periphery, where the cytoplasm is not granular Thus all three phases of maturation are present in one and the same [illegible] stab forms [illegible] the [illegible] nce of toxic granulation in the leucocytes tends to exclude a diagnosis of leukæmia, but Voorhoeve (1938) maintains that toxic changes are found in leukæmia. Kienle also believes that a shift to the left of eosinophils and basophils favours leukæmia, but we have observed a myelocytic shift to the left in all three types of granulocytes in leukæmoid reaction as in Case 20 (p 172) Thaddea and Bakalos (1939) demonstrated predominance of the monocytic reaction in the terminal stages In our experience an increased number of mitoses in myeloid cells favours leukæmia Sternal puncture may be of great value in the diagnosis of aleukæmic forms (Mettier and Purviance, 1937; Henning and Keilhack, 1939; Kimura, 1940; Leitner, 1941; Thaddea, 1943).

Sternal puncture is of considerable importance in the diagnosis of chronic myeloid leukæmia, when qualitative changes are studied (Young and Osgood, 1935, Segerdahl, 1935, Jagić and Klima, 1937, Weil and Perlès, 1940) Abnormal cell divisions, showing the dissociation of nuclear and cytoplasmic division, are important, but these can also be found in leukæmoid reactions and very rarely in infections They may result in bi nuclear and multi nuclear forms. They were particularly frequent in the following case -

PLATE III

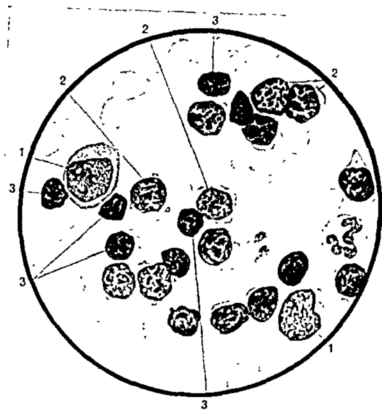
Sternal Marrow in Chronic Myeloid Leukæmia, also illustrating the Normal Leucopoietic Series



1. Myeloblast with a thin rim of basophilic cytoplasm without granules, and fine nuclear meshwork and three nucleoli
2. Promyelocytes with persistent nucleoli.
3. Semimature myelocyte.
4. Mature myelocytes
5. Metamyelocyte
6. Non-segmented polymorph
7. Segmented polymorphs
8. Eosinophil
9. Platelets

PLATE IV

Sternal Marrow in Lymphatic Leukæmia



1 Lymphoblasts with fine nuclear meshwork and nucleoli.
2 Lymphocytes.
3 Senile lymphocytes.

Case 24. L. M , a woman of 59, complained of lassitude, shivering, drowsiness, thirst and sweating since the birth of her third child, at the age of 43. For the last nine years, the spleen had been enlarged, and after one year reached below the umbilicus. There was a feeling of fulness and loss of weight. On examination she was 174 cm tall, 57 5 kg in weight. Proptosis of right eye The left dome of the diaphragm was raised and fixed. X-ray increased left hilar shadows. Apical systolic murmur, blood pressure 140/95 E C G , myocardial damage with inverted T_4. The spleen was firm and reached the pelvis Prominent veins on the anterior abdominal wall Liver enlarged.

Blood. R B C *3 35 millions, Hb 65% = 10 5 g.%;* W.B C. 30,240; myeloblasts 2 5%, promyelocytes 3 5%, myelocytes 14 5%, metamyelocytes 1 [illegible]
eosinophil myeloc [illegible]
basophils 1·5%, [illegible]
per 100 white cells, many twin cells Sedimentation rate (Westergren) 14–35 mm. (1 and 2 hr.).

STERNAL MARROW. Proerythroblasts 0 3, early normoblasts 0 6, normo-



FIG 116 Sternal marrow in chronic myeloid leukæmia with dissociation of nuclear and cytoplasmic division and disturbance of maturation (× 1,050)

[illegible]

The myeloid shift to the left was as pronounced in the sternal marrow as in the blood, but the binuclear myelocytes, metamyelocytes, etc , were more numerous than in the blood (Figs. 116–121). The tendency to nuclear lobation was noticeable and many giant metamyelocytes were present Erythropoiesis was hypoplastic, and here our observations tally with those of Moeschlin and Rohr (1939) Sternal puncture showed abnormal cells similar to those in the peripheral blood These atypical cells indicated a severe hæmopathy in spite of the relatively slight shift to the left and of the presence of many mature leucocytes in the marrow Though the diagnosis was already certain from the blood pictures, sternal

puncture served to confirm it. This case was interesting for its very chronic course. Splenomegaly was present for nine years, but the patient had had symptoms for altogether sixteen years. The actual existence of myeloid leukæmia can, however, be considered certain for the last nine *years only*, when the patient was being treated for "too many blood cells."

The fact that both *bone marrow* and blood showed the same atypically divided bi-nuclear cells would indicate that at least some *of the blood cells originate in the* marrow. According to Rohr (1938) the immature cells in the peripheral blood do not originate from marrow, but from extramedullary hæmopoietic areas (liver and spleen), just as in the fœtus myelocytes and erythroblasts circulate in the blood only as long as hæmopoiesis is predominantly extramedullary, i.e., up to the fourth month. Rohr follows the views of Drinker *et al.* (1922), Doan (1922), Askanazy (1927), Helly (1927) and Orsós (1927) in assuming that the marrow, *in contra-*



FIGS. 117–121. Dissociation of cell division in chronic myeloid leukæmia
Bi nuclear myelocyte, metamyelocytes, stab forms and segmented polymorphs (× 1,050)

distinction to the spleen, possesses a closed vascular system. Consequently marrow cells must reach the blood stream by active movement, i.e., diapedesis. This activity is confined to segmented forms amongst the granulocytes, and to a much lesser degree to the stab forms and juvenile cells, while immature forms do not possess motility of their own. Ro
tions on sternal marrow.
a marrow vessel during r
neighbouring parenchyma showed only more mature cells. In another case the presence of myeloblasts in peripheral blood and the presence *of more mature cells in the* marrow suggested the extramedullary origin of myeloblasts. Thaddea (1943) and we ourselves *have seen cases where the blood showed more immature cells* than the marrow.

Rohr believes that *radiotherapy merely induces profound* changes in the blood picture, but not in the marrow; but Kieule, who states that the *blood picture is a faithful copy of the marrow* picture, does not agree. In Rohr's view myeloid leukæmia remains aleukæmic as long as it is confined to *the marrow*. *Olivari* (1940) has described such a case: a woman aged forty-seven, with persistent aleukæmic chronic myeloid leukæmia, which was *confined*

exclusively to the marrow. We have, however, made repeated observations of myelopathies, in which, after a breach of the marrow-blood barrier, immature cells reached the blood stream without foci of extramedullary hæmopoiesis. Wegelin (1930) observed that it is unreasonable to believe that primitive marrow cells should form extramedullary foci in hæmopoietic organs, if the marrow is at the same time considered to be a hermetically sealed vascular system as Rohr believes. In acute, and also in chronic hæmopathies, therefore, a limited, possibly transient permeability of the marrow-blood barrier may be assumed. The following case of subacute myeloid leukæmia serves as an example, and tends to support Rohr's view —

Case 25. M. A , a farmer of 50, had pains in the loins, especially the left, for three to four years. Feeling of fulness, increasing weakness, loss [illegible] to two years, [illegible] up to four [illegible] four fingers [illegible]

Blood. R.B C. 5 5 millions, Hb. 96% = 15 4 g %, W.B C 14,900, basophils 2 6%, eosinophils 0 3%, myeloblasts 7%, promyelocytes 0 3%, semimature and mature myelocytes 26%, metamyelocytes 9 6%, stab forms 17 3%, segmented polymorphs 23%, lymphocytes 13 3%, monocytes 0 3%, platelets 462,640. Neutrophils showed toxic granulation. Bleeding time forty-five seconds, coagulation time twenty-two [illegible]

[illegible] blasts 0 5, normoblasts 3·5 per 100 white cells; myeloblasts 4%, promyelocytes 2 5%, semimature myelocytes 5%, mature myelocytes 18 5%, metamyelocytes 17 5%, stab forms 10%, segmented polymorphs 18 5%, eosinophil myelocytes 3%, eosinophils 0 5%, lymphocytes 18 5%, monocytes 0 5%, megakaryocytes 0 25%, endothelial cells 0 25%, reticulum cells 1%.

Here the cells in the marrow were obviously more mature than those in the blood, which would favour the extramedullary origin of the myeloblasts and myelocytes in the blood stream. The thickness of the cortex indicated some degree of osteosclerosis, thus making the importance of the extramedullary site of hæmopoiesis plausible.

Among the other marrow findings Jaffé (1933), Ferrata (1935), Fieschi (1936), di Guglielmo (1938), and Kienle (1943) found that the erythroblasts were not diminished in numbers. On the contrary, Kress (1934), Nordenson (1935), Mallarmé (1937), Weil, Isch-Wall and Perlès (1938), Rohr (1940), Thaddea (1943) and we ourselves have found usually rather low figures (see Cases 24, 25) In some cases Fieschi (1936) and Moeschlin (1940) noted an increase in normoblasts. The red blood picture may remain normal for a long time. Polycythæmia may even precede leukæmia, but later, anæmia is almost invariably found Paschkis (1933) and Jaffé (1935) consider that the anæmia is due to hæmolysis rather than to

puncture served to confirm it. This case was interesting for its very chronic course. Splenomegaly was present for nine years, but the patient had had symptoms for altogether sixteen years. The actual existence of myeloid leukæmia can, however, be considered certain for the last nine years only, when the patient was being treated for "too many blood cells."

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FIGS 117–121. Dissociation of cell division in chronic myeloid leukæmia Bi-nuclear myelocyte, metamyelocytes, stab forms and segmented polymorphs. (× 1,050)

distinction to the spleen, possesses a closed vascular system Consequently marrow cells must reach the blood stream by active movement, i.e., diapedesis. This activity is confined to segmented forms amongst the granulocytes, and to a much lesser degree to the stab forms and juvenile cells, while immature forms do not possess motility of their own. Rohr supported this conception by observations on sternal marrow. He has observed masses of myeloblasts in a marrow vessel during a terminal myeloblastic crisis, while the neighbouring parenchyma showed only more mature cells In another case the presence of myeloblasts in peripheral blood and the presence of more mature cells in the marrow suggested the extramedullary origin of myeloblasts. Thaddea (1943) and we ourselves have seen cases where the blood showed more immature cells than the marrow.

Rohr believes that radiotherapy merely induces profound changes in the blood picture, but not in the marrow; but Kienle, who states that the blood picture is a faithful copy of the marrow picture, does not agree. In Rohr's view myeloid leukæmia remains aleukæmic as long as it is confined to the marrow Olivari (1940) has described such a case, a woman aged forty-seven, with persistent aleukæmic chronic myeloid leukæmia, which was confined

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Case 25. M. A , a farmer of 50, had pains in the loins, especially the left, for three to four years Feeling of fulness, increasing weakness, loss of appetite, attacks of sweating, decrease of libido for one to two years, no priapism [illegible] up to four *fingers bread* [illegible] *four fingers* breadth belo [illegible]

Blood. [illegible] B C. 14,900, basophils 2 0%, eosinophils 0·3%, myeloblasts [illegible]%, promyelocytes [illegible] locytes 26%, metamyelocytes 9 6%, [illegible] ymorphs 23%, lymphocytes 13 3%, [illegible] Neutrophils showed toxic granula- [illegible] onds, coagulation time twenty-two minutes (Burker). *Bilirubin direct and indirect negative, serum protein* 8 39%, albumin-globulin ratio 75 : 25, sedimentation rate (Westergren) 21–48 m [illegible]

Ster [illegible] ness of [illegible] blasts 0 [illegible] myelocytes 2 5%, semimature myelocytes 5%, mature myelocytes 18 5%, metamyelocytes 17 5%, stab forms 10%, segmented polymorphs 18 5%, eosinophil myelocytes 3%, eosinophils 0 5%, lymphocytes 18 5%, monocytes 0 5%, megakaryocytes 0 25%, endothelial cells 0 25%, reticulum cells 1%.

Here the cells in the marrow were obviously more mature than those in the blood, which would favour the extramedullary origin of the myeloblasts and myelocytes in the blood stream The thickness of the cortex indicated some degree of osteosclerosis, thus making the *importance of the extramedullary site of hæmopoiesis plausible.*

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reduced erythropoiesis, but we believe, that the depression of erythropoiesis by the leukæmic process itself and replacement of the *erythropoietic tissue by leukæmic cells* are the most frequent causes of anæmia. Weiner and Kaznelson (1926), Dameshek (1935), Klima (1938), Graff (1938), Scott (1939) and de Weerdt (1939) found increased numbers of megakaryocytes, but Helly (1927) in autopsy material reported degeneration of megakaryocytes.

Osteosclerosis, such as in our Case 25, has been observed also by Storti (1937), Graff (1938), Rohr (1940), Erf and Herbut (1944) and Churg and Wachstein (1944). Osteosclerosis is a contraindication to radiotherapy especially when marrow hyperplasia cannot be *confirmed by biopsy, as pointed out by Klima (1938) and* Schartum-Hansen (1940), since it may lead to marrow atrophy and agranulocytosis.

Kienle (1943) has made a *particularly close study of the marrow* during radiotherapy and he distinguishes four types of reaction :—

(1) Approach to normal following irradiation and regression of the leukæmic shift to the left, while erythropoiesis is well preserved. Radiotherapy may be discontinued. Transfusions are not necessary.

(2) Persistently hyperplastic leukæmic marrow with profound disturbance of erythropoiesis, such as hypoplasia, maturation arrest, signs of degeneration and karyorrhexis Radiotherapy must be stopped and blood transfusions should be given at once. Radiotherapy may be resumed when erythropoiesis has recovered

(3) Erythropoiesis healthy, leukopoiesis damaged, with signs of degeneration, karyorrhexis, poor staining qualities of cells, vague contours and coarsening of the nuclear structure Owing to the imminent danger of leukopenia, irradiation should cease, and blood transfusions should be given

(4) Disappearance of erythropoietic and leucopoietic marrow, preponderance of reticulum and plasma cells and fatty marrow These are signs of overdosage of X-rays, which must be stopped Transfusions are indicated.

The increase of mast cells, either reactionary or due to the resistance of these cells to irradiation which may follow radiotherapy, has already been described. When myeloblasts are increased, an unfavourable sign according to Henning (1935), Nordenson (1935), Schulten (1936), Israëls (1936), Klima (1938), and other observers, the dosage of irradiation should be increased, but even then failure is common, as reported by Segerdahl (1935), Henning and Keilhack (1939) and de Weerdt (1939) Recent experience with urethane (1–4 g daily for a minimum of twenty days) has produced satisfactory clinical and hæmatological remissions, very similar to those induced by radiotherapy Lymphatic leukæmia appears to react better than myeloid leukæmia (Paterson *et al*, 1946, Kartagener, 1946, Goodman *et al*, 1946) The last-named group of authors reported improvements in chronic

leukæmias, Hodgkin's disease and lymphosarcoma with Nitrogen mustard, but there is some danger of severe marrow damage with this method.

In the benign "leukæmias" with mature leucocytes (Tushy, 1920; Arneth, 1930; Weil, 1933, Jongh, 1941, Berg 1937), irradiation is superfluous (*leucémie myéloïde à polynucléaires*). According to Thaddea and Bakalos (1939) there is some resemblance to monocytic leukæmia, but it is questionable if these cases are actually true leukæmias.

Eosinophilic leukæmia reported by Hay and Evans (1929), Stephens (1935), Scott (1939), Thomsen and Plum (1939), Bang (1942), Whitby and Britton (1946) and others, and mast cell leukæmia by Joachim (1906), Groat *et al.* (1933), Doan and Reinhart (1941), Casey *et al.* (1946) and others, are special forms of chronic myeloid leukæmia and will not be discussed in detail. In the sternal marrow an increase of eosinophils or mast cells is found. In the former the more common eosinophil leukæmoid reactions must be excluded.

As far as prognosis is concerned, it has already been emphasized that increases of myeloblasts are unfavourable. In the terminal stages the findings may be indistinguishable from acute myeloid leukæmia, as observed by Hadorn (1935), Rietti (1938), and others. When the marrow shows such a degree of immaturity, a chronic course is rare (Millhit, Fouquet and Delort, 1939).

Summary. Though the diagnosis of leukæmic forms of chronic myeloid leukæmia can be established from the blood picture, sternal puncture gives valuable information in the diagnosis of aleukæmic forms and is also of value for prognosis and as a control to therapy.

CHRONIC LYMPHATIC LEUKÆMIA

As lymphatic leukæmia affects the lymph nodes and the spleen predominantly, a characteristic picture would not be expected in the marrow. However, Weiner and Kaznelson as early as 1926, demonstrated considerable lymphocytosis in sternal trephine preparations. This has been confirmed by all subsequent workers. Such lymphoid hyperplasia originates from the marrow germ centres previously observed by Askanazy ([illegible]). In agreement with Nordenson (1935), Rohr (1935), Klima (1938), Thaddea (1943) and Kienle (1943) we have found that the number of mitotic figures was usually low. Early cases where marrow findings were far from definite have been reported by Weiner and Kaznelson (1926), Nordenson (1935), Schulten (1936), Klima (1938), Bach (1939) and Heilmeyer (1942), but most workers agree with Rohr (1935), Klima and Seyfried (1937) and Tischendorf and Herzog (1940), that sternal puncture is usually of diagnostic value in such early

cases. One case, a man of fifty-eight years, has so far been the only one we have seen with a normal figure (9·5%) for lymphocytes, otherwise our figures have always been high. Even if the lymphocytes are only slightly increased in slowly progressing cases and in the initial stages, as pointed out by Vischer (1938), nevertheless lymphatic leukæmia is a possible diagnosis because there are very few diseases accompanied by an increase in lymphocytes in the marrow; infectious mononucleosis is one (Israëls, 1939), but this is easily diagnosable on other grounds. In the later stages the marrow findings become more definite. In a case of subacute lymphatic leukæmia Klima found up to 75% lymphoblasts in the sternal marrow. Holmes and Broun (1933) report as many as 95% lymphoblasts and 5% lymphocytes, but this observation, as Schulten (1936) states, may be due to a difference in nomenclature, especially as these American authors also record 2·5% megaloblasts. Klima (1938) in 12 subleukæmic and aleukæmic cases found between 50%–80% lymphocytes and lymphoblasts; and in 8 cases, normal or only slightly increased numbers of lymphoid cells; de Weerdt (1939) found more than 64% of lymphoid cells in all his 6 cases. As stated by Willi (1936), de Weerdt and others, lymphoblasts predominate in advanced cases and atypical forms with lobulated nuclei and Rieder forms as well as maturational disturbances occur. In our experience even in advanced cases there are always plenty of lymphocytes as well as lymphoblasts, usually in equal numbers, and sternal puncture may give much useful information in aleukæmic forms. Henriksen (1941) based his diagnosis exclusively on sternal puncture Engelbreth-Holm (1939) and others report that aleukæmic and subleukæmic forms are particularly frequent in infancy. Lymphatic transformation of the marrow frequently establishes the diagnosis in these cases (Nordenson, 1935; Fleischhacker and Seyfried, 1937; Vogel, Erf and Rosenthal, 1937; Gingold, 1938, Roelsen, 1939, Megassini, 1940; Wintrobe and Mitchell, 1940; Hynes, 1940, Leitner, 1941) Neumark (1947) has described a case of erythrodermia in lymphatic leukæmia, in which there were many mitotic figures in the peripheral blood, a very rare phenomenon in lymphatic leukæmia. There were 75% of lymphoid cells in the marrow and 75% in the blood with a total white cell count of 39,000 The following case is an instructive example for the diagnostic value of sternal puncture in aleukæmic lymphatic leukæmia :—

Case 26. R. W., a 45-year-old farmer, had suffered from lassitude, visual disturbances, loss of appetite, abdominal swelling and enlarged inguinal, supraclavicular and axillary glands for one year When examined his abdomen was protuberant, the spleen reached below the

= 14 2 g %, WBC
ed polymorphs 48%.

lymphocytes 32%, monocytes 3%. The patient was admitted with the diagnosis of Hodgkin's disease on account of the eosinophilia.

STERNAL MARROW. Proerythroblasts 1·5, early normoblasts 3, normoblasts 30 per 100 white cells; myeloblasts 0·75%, promyelocytes 1%, semi-mature myelocytes 0·25%, mature myelocytes 14%, metamyelocytes 13%, stab forms 15·75%, segmented polymorphs 17%, eosinophil myelocytes 2·75%, eosinophil metamyelocytes 4·5%, eosinophils 3·25%, lymphocytes 32%, monocytes 2·5%, megakaryocytes 1%, endothelial cells 0·25%, plasma cells 0·5%, lymphoid reticulum cells 1%; mitoses not increased.

The lymphocytes in the sternal marrow were predominantly mature forms, but because 32% represents about four times the normal, the diagnosis of lymphatic leukæmia could be made and a gland removed by biopsy confirmed it.

Of special interest are the cases where the lymphatic hyperplasia, or rather metaplasia, exclusively affects the marrow. Such cases have been reported under the name of "lymphadenia ossium" by Runeberg (1883), Litten (1898), Rubinstein (1901), Senator (1904) and Domarus (1937). Kienle (1943) quite rightly points out, however, that histological diagnosis frequently becomes very difficult owing to the rapid post-mortem changes in the leukæmic marrow. To date, three relevant cases have been diagnosed by marrow biopsy. In Rohr's (1940) case and that of Klima and Seyfried (1937) the increase of lymphocytes in the blood (80%) clinched the diagnosis, while the lymphocyte percentage was only 40% in the case described by Storti (1937). The latter described large lymphoblasts with primitive nuclei and nucleoli and the coarse azure granules in the cytoplasm usually only seen in mature cells (dissociation of nuclear and cytoplasmic maturation).

In the following case the disease was confined to the marrow :—

Case 27. G. F., a man of 50, had appendicectomy 1907, right iridocyclitis in 1937 and an operation for hernia early in 1942. For the past eighteen months he had suffered from tiredness, weakness, feeling of fulness and pallor. When examined he was 166 cm in height, 65·5 kg in weight, spleen not enlarged, lungs normal, X-rays of chest did not show any enlargement of hilar glands. Lymph glands not enlarged, except in the left axilla, where there was a small gland palpable.

BLOOD. R.B.C. 3·8 millions, Hb 80% = 12·8 g %, C.I. 1, W.B.C. 32,400; basophils 0·1%, eosinophils 0·5%, stab forms 0·8%, segmented polymorphs 3·2%, lymphoblasts 1%, lymphocytes 93·9%, monocytes 0·5%. Many ghost cells; reticulocytes 2·3%, platelets 280,000; bleeding time one minute (Duke), coagulation time seven minutes (Milian). Sedimentation rate (Westergren) 2–8 mm (1 and 2 hr.). Repeated blood pictures gave similar results.

STERNAL MARROW. Proerythroblasts 1, early normoblasts 2·3, normoblasts 28·6 per 100 white cells; myeloblasts 1·6%, promyelocytes 2%, semi-mature myelocytes 4·3%, mature myelocytes 12%, metamyelocytes 11%, stab forms and segmented polymorphs 14·3%, eosinophil myelocytes 0·6%, eosinophil metamyelocytes 1%, eosinophils 0·3%, lymphoid cells 51%, monocytes 0·3%, megakaryocytes 0·3%, proplasmocytes 0·3%, plasma cells 1%, lymphoid reticulum cells 0·6%, phagocytic reticulum

cells 0 3%, endothelial cells 0 3%, fat cells 0·3%. Apart from the cells counted, groups of lymphocytes were apparent, and in histological sections predominated (Fig. 122).

Despite the absence of splenomegaly and adenopathy (with the exception of an axillary gland), sternal puncture showed an appreciable increase of lymphoblasts and lymphocytes which in places dominated the picture completely (Fig. 122).

In agreement with the authors quoted, we have found an increase of lymphocytes in the marrow in all our 7 cases in the ordinary leukæmic forms of lymphatic leukæmia. The number varied between 32%–80%. Most of the lymphocytes were mature, but in advanced cases lymphoblasts dominated the field. In early cases, as far as the other cell systems were concerned, there was normal granulocytopoiesis and erythropoiesis. In advanced leukæmias

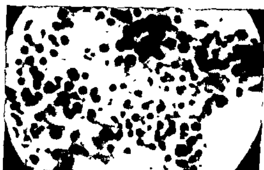


Fig 122 Purely lymphoblastic lymphocytic marrow in lymphatic leukæmia localised to the marrow Histological section of sternal puncture material (×500)

with considerable lymphatic hyperplasia, granulocytopoiesis was depressed and figures for normoblasts were low In cases such as that of Feuchtinger (1942) which was complicated by hæmolytic anæmia, the figures for normoblasts may be raised In Bertoni and Spezie's (1942) case, myeloblastic hyperplasia was found, but it appears doubtful if this case should be interpreted as lymphatic leukæmia The megakaryocytes do not show any particular changes.

Owing to lymphatic marrow hyperplasia, osteoporosis may occur, as reported by Markoff (1939) The danger of lack of homogeneity of the marrow must be watched for, particularly in these cases. Escudero and Varela (1928) in 2 cases found normal marrow in the tibia, while sternal marrow biopsy showed an increase in the lymphocytes In assessing the prognosis sternal puncture gives valuable guidance, and cases with immature lymphoblastic marrow must be viewed gravely.

In the control of radiotherapy also, sternal puncture is useful According to de Weerdt (1939), lymphatic leukæmias with lymphoblastic marrow are radio-resistant. Kienle (1943) emphasizes the observation of the numbers and morphology of the

normoblasts. Normoblastic hypoplasia and signs of degeneration such as karyorrhexis and mitotic disturbances demand cessation of radiotherapy, as would also aplasia of the granulocytic system. The lymphatic metaplasia of the marrow persists even after irradiation.

Summary. Sternal puncture is a valuable aid to diagnosis in lymphatic leukæmia, since even in the early stages of the disease it will show at least a moderate increase of lymphocytes, and in the late stages it will show a considerable increase of lymphatic cells with lymphoblasts and atypical forms.

THE ACUTE LEUKÆMIAS

Stem Cell Leukæmia (*Leucémie à cellules souches*)

Though research in morphology has made tremendous progress, there are still cases where it is impossible to differentiate with certainty whether the leukæmia is of the myeloid or of the lymphatic type, because the cells are too primitive and the leukæmic process affects the earliest stages of cell development As the oxidase reaction is negative in these primitive cell forms, diagnosis cannot be established by this method. Histological sections are often necessary to settle the diagnosis There are, however, cases which exhibit both myeloid and lymphatic proliferation In some myeloses there are not only collections of small lymphocytes, but definite lymphomata. Sučić (1937) considers such proliferation of the other system to be a reactionary hyperplasia and speaks of "mixed leucoses." Hadorn (1935) also reports the simultaneous occurrence of paramyeloblasts and paralymphoblasts, but he admits that the distinction of these most primitive forms is very difficult He therefore prefers the term "paraleucoblast." Simultaneous myeloblastic and lymphoblastic leukæmia has also been described by Gandellini (1939) Cases with cells which are not differentiated are often termed *stem cell leukæmia* (Kwasniewski and Henning, 1926; Coste, 1934, Hadorn, 1935, and Jordan, 1937) The hæmocytoblastic leukæmias of Pappenheim (1914), Ferrata (1921), di Guglielmo (1925), Fieschi (1936), Quattrin (1941) and Kienle (1942) do not actually belong to this group, because the hæmocytoblast of Ferrata corresponds to our myeloblast, and such cases, therefore, are acute myeloblastic leukæmias in our terminology. It is probable that most cases described hitherto as stem cell leukæmia, were in fact acute myeloid leukæmia, in which the diagnosis could not be made with certainty on morphological grounds. Strictly speaking, the term stem cell leukæmia should be applied only when stem cells capable of multi-potential blood cell formation, i e., hæmohistioblasts, are affected.

Acute Myeloid Leukæmia (Myeloblastic Leukæmia)

The problem of *acute myeloid leukæmia* bristles with controversy. In agreement with Pappenheim (1914), Hirschfeld (1925), Schilling (1925), Muller and Sprohnle (1929), Naegeli (1935), Cecconi and Micheli (1937), Tschopp (1939) and others, we regard acute myeloid leukæmia as a peculiarly progressive form of the chronic type of myeloid leukæmia. The reasons for this opinion will be discussed later. According to other authors, notably Ferrata (1935), acute myeloid leukæmia is a disease in its own right, and therefore independent of chronic myeloid leukæmia. Hoff (1935), Henning (1936), and Voit and Landes (1938), believe that acute myeloid leukæmia represents a reaction to infection or other noxious agents, and Arneth (1936) holds that, like leukæmia in general, it is merely a reaction. Fraenkel (1895) considered that in the past too many cases were diagnosed as lymphatic leukæmia and Ziegler (1906), Naegeli (1931), Rohr (1940) and Thaddea (1943) now regard all acute leukæmias as myeloid in type. Arneth believes them to be lymphatic leukæmic reactions, which, as far as infantile cases are concerned, is admitted by Naegeli, and Willi (1936). These divergent opinions indicate the great difficulty in differential diagnosis. Oestreich's (1931) case illustrates this problem particularly well. Naegeli himself diagnosed this case as myeloid leukæmia, but autopsy produced the diagnosis of lymphatic leukæmia. We would list the signs in blood and marrow for the differential diagnosis as follows :—


FIG 123 Bone marrow *reticulum cell with Auer rods in panmyelopathy* (× 1,000)

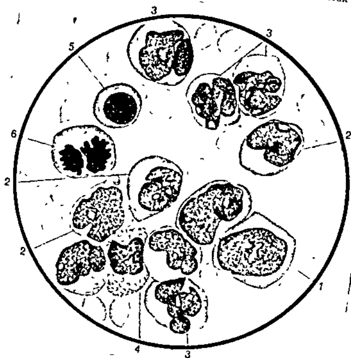
The presence of the more mature cells, usually found in the acute leukæmias, if only in small numbers, may help in the diagnosis In the very primitive forms the oxidase reaction does not help, as noted by Hirschfeld (1925), Naegeli (1931), Watanabe (1933) and Leitner (1935)

The hiatus leukæmicus of Naegeli (i e , the absence of intermediate forms between myeloblasts and mature neutrophils) would favour myeloid leukæmia. We have, however, observed this phenomenon in leukæmoid reactions too, though it was transient The hiatus is usually not complete. Like Pittaluga (1941), we have also found promyelocytes, in addition to myeloblasts, substantiating the diagnosis

The little azurophil rods, described by Auer (1906), in the primitive cells, favour a diagnosis of myeloblasts Rietti (1938), Penati and Momigliano-Levi (1934) have, however, observed them also in lymphatic and monocytic leukæmia. Arneth (1939) found them particularly plentiful in a case of lymphatic leukæmia

PLATE V

Sternal Marrow in Acute Myeloid Leukæmia (Myeloblastic Leukæmia)



1 Myeloblast with large nucleoli
2 Myeloblasts with slight lobation (Paramyeloblast)
3 Myeloblasts with marked lobation (Paramyeloblast)
4 Semimature myelocyte
5 Early basophilic normoblast.
6 Myeloblast in mitosis

and Steinmann (1939) in our hospital in a case of plasmocytoma. We have also seen them in reticulum cells in a case of panmyelopathy confirmed by autopsy (Fig. 123).

Observation of mitotic figures is often of value. Mitoses are much rarer in lymphatic leukæmia than in myeloid leukæmia, as reported by Schulten (1936), Leitner (1941) and Thaddea (1943) (*but see Neumark, p. 208*). *Stephens (1937) and Leitner (1941)* found the angle of mitosis valuable as it varies in the different cell forms, being 68°–69° for myeloid, and 38°–42° for the lymphatic variety. Chromosomes, however, may show numerous aberrations. Their number is often only half the normal, i.e., 24 instead of 48, and it may be an odd number (Groat, 1933, Moeschlin and Rohr, 1939).

TABLE 10

Myeloblastic Leukæmia (Case 28)

Date	8.5	18.5	26.5	29.5	4.6	9.6	14.6	17.6
Leucocytes per cmm	153,000	105,000	210,000	172,000	226,000	205,000	150,000	19,000
Myeloblasts	98%	67%	91.6%	77.5%	85%	85%	90.3%	56%
Promyelocytes	1%	7%	—	3%	4.6%	6%	3.3%	3.5%
Segmented polymorphs	0.5%	15.3%	1.6%	1%	1.3%	7%	4.6%	38.5%
Lymphocytes	—	2%	—	—	—	0.6%	0.3%	1%
Monocytes	0.5%	0.3%	0.3%	—	—	0.3%	0.3%	1.5%
Erythrocytes, millions per cmm.	1.5	1.62	1.15	1.3	1.23	1.44	0.96	0.76
Hæmoglobin (100% = 16.0 g %)	24%	28%	18%	22%	22%	22%	22%	16%
Colour index	1.11	1.15	1.24	1.13	1.1	1.3	1.15	1.32
Blood sedimentation (mm 1 and 2 hr.)	—	—	—	—	—	178/180	—	177/180

The presence of paramyeloblasts is of no value in diagnosis, as paralymphoblasts are too similar in appearance. Heilmeyer (1942) points out that paramyeloblasts with whitish nucleoli and lymphoid reticulum cells are often characteristic.

The number of nucleoli may be helpful, because cells with only one nucleolus are more common among the lymphoblasts, while among the myeloblasts, cells with two or more nucleoli are more numerous (Lambin, 1938, de Weerdt, 1939, Leitner, 1941).

In 2 cases of acute lymphatic leukæmia observed by us (one was published in 1935), the cells could be distinguished definitely from myeloblasts on morphological grounds. Their chromatin structure was much more compact, and with their trabecular arrangement they were more reminiscent of lymphocytes. More primitive forms with a fine meshwork of chromatin were also seen, but the classification of these lymphoid cells made possible a diagnosis of acute lymphatic leukæmia, subsequently confirmed by autopsy.

Many reports of sternal marrow in acute myeloid leukæmia

are found in the medical literature. Jackson, Parker, Robb and Curtis (1931), Leitner (1935), Fieschi (1936), Schulten (1936), Klima (1938), de Weerdt (1939), Henning and Keilhack (1939), de Filippi (1940), Rohr (1940), Quattrin (1941), Kienle (1942), Thaddea (1943), and others have described myeloblastic or hæmocytoblastic marrows. We have found, as have many other workers, that sternal puncture is an invaluable aid to diagnosis in the aleukæmic forms. Kaplan and de la Chevasnerie (1940) were able to make a diagnosis by sternal puncture in a case presenting the picture of aleukia. Evensen and Schartum-Hansen (1941) collected 22 cases of aleukæmic myeloblastic leukæmia, the diagnosis in many of which depended on sternal puncture. The initial stages are usually aleukæmic, but Lübbers (1942) states that leukæmic and aleukæmic stages may alternate or follow each other. There is no basic difference between subacute and acute myeloid leukæmia. In Evensen and Schartum-Hansen's series the duration of the disease was 3–5 months; the younger the patient the shorter the duration. The following case of subacute myeloid leukæmia cannot be distinguished hæmatologically in any way from acute leukæmia:—

Case 28. F. A., 54, an engineer, was operated on for a strangulated inguinal hernia in November, followed by an attack of phlebitis. In *January he started work again, but later had to discontinue owing to* abdominal pains, weakness, loss of appetite, headache, vomiting, fever (sometimes up to 104° F.). Marked loss of weight. P.H.: 1920 anæmia, 1935 influenza and nephritis (Hb. 64%–69% = 10·3–11·1 g.%). On examination: temperature up to 105° F., marked splenomegaly and hepatomegaly, pallor, evidence of loss of weight. Urine: 0·05% albumen.

Blood: R.B.C. 1·35 millions, Hb. 24% = 3·9 g.%, W.B.C. 153,000, myeloblasts 98%, segmented polymorphs 0·5%, monocytes 1·5%, platelets 73,000, reticulocytes 1·4%. Poikilocytosis, anisocytosis, polychromasia. Serum uric acid 14·3 mg.%, cholesterol 68 mg.%, blood urea 63 mg.%. Repeated epistaxis [illegible]

myeloblasts, especially initially, had round nuclei, but [illegible] with a much lobated nucleus and immature cytoplasm which gave a negative oxidase reaction were also present.

The illness progressed rapidly. During a stay of five weeks in hospital the blood was examined fourteen times, showing a successive reduction of the number of leucocytes to 19,000. Anæmia increased and finally [illegible] The [illegible]

myeloblastic leukæmia. The blood findings are [illegible]

This is a case of subacute myeloid leukæmia with 98% myeloblasts in the blood, hiatus leukæmicus, paramyeloblasts, profound anæmia, thrombocytopenia, *hæmorrhagic diathesis* with bruising even after

the slightest trauma, and high fever. In the marrow (Fig 124) there were myeloblasts, stem cells, no intermediate stages in the granulocytic series and aplasia of erythropoiesis and thrombopoiesis. The number of leucocytes decreased from 153,000 to 19,000 and the spleen became smaller at the same time. This fact favours Rohr's view that with the decrease in size of the extramedullary hæmopoietic tissue the numbers of myeloblasts fall.

Most authors agree that normal myeloblasts are rare in acute myeloid leukæmia. Atypical cells, paramyeloblasts, usually dominate the blood and marrow pictures, and the cells are often very pleomorphic. According to Rohr (1940) each case of acute leukæmia has its own cell type. Among the paramyeloblasts he describes: micromyeloblasts; promyelocytoid forms; monocytoid forms; highly pleomorphic forms.

Though paradoxical, it might be stated that atypical cells are typical of myeloblastic leukæmia. The following case will illustrate the cell pleomorphism and the diagnostic importance of sternal puncture. It is a case of subleukæmic myeloblastic leukæmia:—



FIG 124 Myeloblastic marrow in acute myeloid leukæmia with hiatus leukæmicus (×500)

Case 29. S H, a man of 29, was admitted on December 23rd, 1940, with open right-sided pulmonary tuberculosis. Blood sedimentation rate (Westergren) was 38–61 mm (1 and 2 hr). On January 2nd, 1941, a right artificial pneumothorax was induced and completed with cauterizing apicolysis in May and September, 1941. His condition improved. The sputum became negative in March, 1941, blood sedimentation rate returned to normal. The patient was due to be discharged as cured in the middle of November, 1941, but the sedimentation rate rose to 48–76 mm, without any clinically recognizable cause. On closer questioning, however, we elicited that the patient recorded his temperatures too low. Supervised temperature taking showed pyrexia of 103° F. The red blood picture showed nothing remarkable (Hb 90% = 14 4 g %, R B C 4 73 millions, C I 0·96), leucocytes were 5,000, of which 68 5% were peculiar monocytoid cells. The medical officer in charge of the case, Dr Kaiser, sent me blood films for diagnosis, and later marrow smears. The provisional diagnosis, made at first at the sanatorium, was lymphoid-celled glandular fever, but this was excluded on the blood picture which showed myeloblastic leukæmia.

The following observations were made on the blood picture. (1) The white blood picture was dominated by large primitive cells with bizarre lobation of the nuclei, which often showed large nucleoli. (2) Some of these cells gave a positive peroxidase reaction. It was therefore established that they belonged to the neutrophil series. (3) There was a definite hiatus leukæmicus, and apart from segmented forms there were only very few stab forms present. (4) There were immature eosinophil cells, some with lobed nuclei, with immature, multicoloured or grey-blue granulations, and there were immature basophil cells, and the number of basophil cells

was raised. (5) There was anisocytosis and polychromasia of erythrocytes and thrombocytopenia.

STERNAL MARROW. Early basophilic normoblasts 0, normoblasts 2·25 per 100 white cells; myeloblasts and paramyeloblasts 91·75%, eosinophil myelocytes 3·25%, basophils 2 25%, reticulum cells 2·75%. Many cells with bizarre nuclei (Fig 125)

The following notes were kindly supplied by Dr. Kaiser: At the onset, the spleen or liver were not enlarged, but by percussion the area of splenic dulness reached the anterior axillary line. The patient did, however, even then have pains in the splenic region The right axilla contained one and the left axilla two enlarged glands In December hæmorrhages appeared in the skin and mucous membranes of the cheeks, gums and tonsils and the patient had to be admitted to hospital He deteriorated rapidly with an ulcer at the left corner of the mouth, increasing rapidly in size and depth. Finally marked hepatomegaly and splenomegaly developed. The patient died on December 30th, 1941. The blood picture is set out in Table 11.

TABLE 11

Myeloblastic Leukæmia (Case 29)

Date	23 6 Last blood picture before leukæmia	11 11	14 11	15 11	21 11	2 12	10 12	24 12
R B C. million per cmm.				4 73		4 47	3 09	2 2
Hb. (100% = 16 g.%)				90%		91%	77%	50%
W B C. per cmm			5,300	5,000	8,800	21,000	48,800	86,20
Micromyeloblasts %							5 6	3 7
Lobed myeloblasts %		63 5	64 0	68 5	65 5	75 5	78 6	74 3
Promyelocytes %							5 6	3 7
Stab forms %	7 0		1 5					2 0
Segmented polymorphs %	46 5	11 5	5 0	4 0	3 0	2 0	4 3	5 3
Eosinophil myelocytes %							0 6	
Eosinophils %	1 5	0 5	1 0	0 5	1 5	0 5	1 6	0 3
Basophils %	1 0				0 5	0 5	3 6	1 7
Lymphocytes %	36 5	24 5	28 5	27 0	26 5	21 5	5 0	8 3
Monocytes %	7 5							0 3
Platelets per cmm							45,750	33,700
Reticulocytes %								2 3%
Sedimentation rate (Westergren, 1 and 2 hr in mm)	2/12	48/76					78,106	134,14

FINDINGS AT AUTOPSY. Acute myeloblastic leukæmia. Marked · (2,780 g). Marked red ly nodular, partly diffuse is of similar infiltrations in region of lower ileum, cæcum and ascending colon. Hæmorrhagic diathesis. Multiple pericardial, pleural, laryngeal petechiæ, as well as in the serous coat of the gut, and in the right renal pelvis. Profound general anæmia

This case showed several interesting features First of all the patient was under medical supervision before the onset of leukæmia, and the beginning of the disease could be defined accurately

In blood smears the extreme lobation of the primitive cells in the peripheral blood as well as in sternal marrow (Fig. 125) was noticeable. The diagnosis could be established from the onset on account of various hæmatological peculiarities, though the disease began in an aleukæmic phase. All three varieties of granulocytes were involved; the primitive cells had large nucleoli and some gave a positive oxidase reaction; there was thrombocytopenia and a normoblastic crisis. There was no anæmia at the beginning Considerable splenic and hepatic enlargement developed terminally, and the myelosis became frankly leukæmic (86,200 white cells), which once more indicates the importance of extramedullary hæmopoiesis. Especially interesting was the presence of open pulmonary tuberculosis, as tuberculosis has been indicted by various authors, especially Arneth, as a cause of leukæmia. We shall discuss this question when we deal with leukæmoid reactions (pp. 234–243)

Tuberculosis may well change the character of leukæmia, as in the cases described by Kruckemeyer (1940), Bichel (1942), Nielsen (1942) and others, in which the clinical and hæmatological aspect favoured acute myeloid leukæmia, but at autopsy no evidence of leukæmic changes could be detected We do not consider that the diagnosis of leukæmia was established in these cases just because in other similar cases leukæmic changes were actually found at autopsy, as for instance in a case of acute generalized tuberculosis, reported by Heilmeyer (1942), where panmyelophthisic anæmia was diagnosed clinically (leucocytes, 1,300), and in cases recorded by Quincke (1902), Hemmerling and Schleussing (1927) and others The case just reported provides evidence against the ætiological importance of tuberculosis, since leukæmia only began after the tuberculous process had become inactive, and tuberculosis did not progress even after the onset of the leukæmia.



Fig 125 Sternal marrow with bizarre paramyeloblasts in acute myeloblastic leukæmia (× 1,400)

We have already mentioned that clinical and hæmatolog observations seem to indicate some connection between acute a chronic myeloid leukæmia In the terminal stages of chro leukæmia the same symptoms can be seen as in acute leukæmia hæmorrhagic diathesis, anæmia, pyrexia, predominance of myelo blasts in blood and marrow, hiatus leukæmicus and paramyeloblasts (Hadorn, 1935, Rietti, 1938, Tschopp, 1939) It is even possible that some of the supposedly acute leukæmias are in fact

merely terminal stages of chronic leukæmia which had not been recognized. Chronic myeloid leukæmia may remain symptom-free for long periods; in a case reported by Weitz (1938), for six years. In Case 29 (p. 215) long duration could be excluded by the previous blood examinations. Kienle (1942) has observed by marrow biopsy true remissions in a case of acute myeloid leukæmia. He has thus made an important contribution to the conception of the identical pathogenesis of acute and chronic leukæmia. In this case with 100,000 leucocytes in the blood, 90% of which were myeloblasts, the sternal marrow showed 80%–90% myeloblasts. Three weeks later a remission occurred. There were no myeloblasts in the blood but only mature leucocytes (55,000). Sternal marrow showed only 1·8% myeloblasts, but also promyelocytes and myelocytes and 32% mature neutrophils. The only evidence of persisting leukæmia were the Auer rods. Two weeks later sternal puncture showed once more a myeloblastic marrow (70%) while the blood continued to contain mainly mature forms, and only 3% metamyelocytes and 1% myelocytes. A few days later the remission in the blood came to an end also and blood smears once more showed 90% myeloblasts. We have quoted this interesting case fully because it provides evidence against Rohr's view of the release of primitive myeloblasts from the spleen into the blood stream. It also confirms the fundamental and primary position of the bone marrow in myeloid leukæmia.

Similar cases of leukæmia with remissions have been reported before, but marrow biopsies have only been performed in the cases of Jackson *et al* (1931), Riccitelli (1934), Henning (1936), de Weerdt (1939), Bernard (1939), de Fillippi (1940), Szonell (1940), Evensen and Schartum-Hansen (1941), Moeschlin (1943), Roth (1943). In Henning's case the marrow was examined during an exacerbation as well as a remission, Jackson *et al*'s case only in the second crisis, and Szonell's and Evensen and Schartum-Hansen's cases in a crisis. Riccitelli's case had a sternal puncture in the first crisis only. In none of these cases, with the exception of Henning's case, is there any proof of marrow remission. De Weerdt, however, examined his case carefully with repeated marrow biopsies. During the course of 7 months two remissions occurred, the first lasting 3 months and the second though incomplete, lasting 10 days. In the first remission the myeloblasts disappeared completely from the blood, their number in the marrow fell at first from 97·2% to 1·3%, and later they disappeared completely. Bernard observed only one remission in his case and the marrow showed exclusively undifferentiated primitive cells during crisis, and mature myelocytes during remission. De Fillippi's case behaved similarly, but Moeschlin reported a case with three remissions of long duration and a second case with incomplete remissions, as judged by the marrow. In his first case, which ended fatally after sixteen months, both blood

and marrow pictures repeatedly became almost normal, and during a remission the patient was free from symptoms and able to follow his usual occupation. In his second case the remission was only of short duration, and a fortnight later the marrow was once more almost exclusively infiltrated with pathological cells. In the case recorded by Roth the development of the remission was observed four times by four sternal punctures. Schaefer (1926), Brogsitter and Kress (1930), Bock (1932) and Marcus (1936) reported cases with remissions complete from the clinical and hæmatological points of view, but they were not examined by sternal puncture, and it is therefore impossible to know whether the marrow showed improvement also The same applies to the incomplete remissions in which splenomegaly persisted, reported by Micheli and Penati (1933), Colarizi (1935) and Lambin (1937). Hemmeler and Jéquier-Doge (1944) described 5 cases with remissions varying in degree and duration These cases show transitions particularly well and favour the theory of close relationship of acute, subacute and chronic myeloid leukæmia

The question arises, are these cases an argument against the neoplastic nature of leukæmia? Moeschlin denies this, pointing out remissions in malignant tumours following changes in the defence mechanism of the body against the tumour, due to infection or other circumstances (Frauchiger, 1929, Taylor and Alsop 1937). We do not know whether such total remissions occur in malignant tumours. If they do, they must be of an extreme rarity. We believe that remissions in myeloid leukæmia indicate the need for a special place for the leukæmias in a nosological system, they cannot be included easily with the system of known tumours or infections Roth (1943) concludes from his observations that acute myeloid leukæmia is either curable or can undergo remission and then cannot be recognised hæmatologically; his alternative is that spontaneous cure may occur, but later on a new disease may arise following the occurrence of the same circumstances The cases quoted here appear to favour the theory of remission Recurrence was observed in all cases followed up for a sufficiently long time, in a case of Schittenhelm's (1926) after as much as one year The findings in total remissions of myeloid leukæmias not only emphasize the importance of sternal puncture for diagnosis and prognosis pointed out especially by Kienle, but also shed light on the problem of the relationship of acute and chronic myeloid leukæmia. Acute myeloid leukæmia in remission may show the hæmatological picture of a chronic form, and in relapse may return to the myeloblastic stage Acute, subacute and chronic leukæmias are stages or forms of one and the same disease This is supported also by animal experiments (Storti, 1940), in which implantation produced in one animal acute leukæmia, in another the chronic form and in others mixed forms Though in infections and intoxications, pictures have

been seen which are very similar to acute myeloid leukæmia clinically and hæmatologically, the identity of these processes with leukæmia is not proved.

In acute myeloid leukæmia another important marrow finding is the hypoplasia of erythropoiesis, which we have observed constantly. Megakaryocytes were diminished or absent in our cases and were never increased in numbers. The primitive reticulum cells were often increased, but plasma cells were *invariably diminished*. Because agranulocytosis and aleukia often show an increase of plasma cells their diminution in acute myeloid leukæmia is important in differential diagnosis.

Summary. Sternal puncture is often of great importance for the diagnosis and prognosis of acute myeloid leukæmia. Studies of sternal marrow have contributed to a clearer understanding of medullary and extramedullary hæmopoiesis and to the problem of the relationship between acute and chronic myeloid leukæmia.

Acute Lymphatic Leukæmia (Lymphoblastic Leukæmia)

The existence of acute lymphoblastic leukæmia is still contested; Naegeli (1931) recognizes such a condition only in infancy. Willi (1936) in infantile cases of lymphatic leukæmia has observed a predominance of lymphoblasts and paralymphoblasts, *i.e*, lymphoblasts with indented or lobulated nuclei, in sternal marrow. However, undoubted cases of acute lymphatic leukæmia have been described in adults (Gosio, 1929; Bowcock and Bishop, 1930; Moore and O'Farrel, 1931; Krumbhaar and Custer, 1935; Leitner, 1935; Stasney and Downey, 1935; Klima and Seyfried, 1937; de Weerdt, 1939; Heilmeyer, 1942). In both cases published by de Weerdt the sternal marrow consisted almost entirely of lymphoblasts with one to two nucleoli. Though Heilmeyer found that many cases designated acute lymphatic leukæmia could be shown by hæmatological and sternal marrow examination to be cases of myeloblastic leukæmia, he also reported definite cases of acute lymphatic leukæmia, which like a case reported by us (Leitner, 1935), were confirmed by autopsy Rittmann (1934) reported a case as acute lymphatic leukæmia which simulated agranulocytosis and led to death so rapidly that there was insufficient time for the development of the typical extramedullary changes We regard the diagnosis of such cases, however, as uncertain, because in so controversial a disease, the diagnosis should only be made when all the clinical, hæmatological and morbid anatomical findings are typical.

It is still uncertain how far sternal marrow examination will help us to understand this problem Our own observations agree with those of Stasney and Downey (1935), Klima and Seyfried (1937), de Weerdt (1939), Heilmeyer (1942), and other authors

and show a considerable increase of lymphoblasts in the sternal marrow. Sapinski (1942) published a case of acute lymphatic leukæmia with extensive localization in the bone marrow, such as we have observed in chronic lymphatic leukæmia (Case 27, p. 209).

The following case illustrates the importance of sternal puncture in acute lymphatic leukæmia :—



FIG 126.

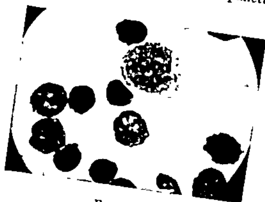


FIG 127



FIG 128

FIG 126 Lymphoblastic marrow in acute lymphatic leukæmia (× 500)
FIG 127 Lymphoblastic lymphocytic marrow in lymphatic leukæmia (× 1,050)
FIG 128 Lymphoblastic lymphocytic marrow and smear cells in lymphatic leukæmia. (× 1,050)

Case 30 H R , a man of 20, one month previously felt ill with lassitude, weakness, pallor and swelling of lymph glands He had had pleurisy one year before, which incapacitated him for three months, followed by complete recovery On examination he was very pale, there were large, dense groups of glands in both sides of the neck, liver and spleen were enlarged Loud systolic murmur over the mitral area Pyrexia up to 101 5° F

BLOOD R B C 1 25 millions, Hb 30% = 4 9 g %, C I 1 3, W B.C. 4,450, myelocytes 2%, metamyelocytes 3%, stab forms 16%, segmented polymorphs 13%, lymphoblasts 3%, lymphocytes 59%, monocytes 1% Platelets normal, reticulocytes 0 1%, anisocytosis, poikilocytosis, polychromasia Bleeding time 2 min 15 sec, coagulation time 4 min, sedimentation rate (Westergren) 130–163 mm (1 and 2 hr)

STERNAL MARROW. Early basophilic normoblasts 0, normoblasts [illegible]

and 49 5%, with about 7·5% lymphoblasts. The lymphoblasts in the marrow were *oval or round and had one or two definite nucleoli* The nuclear chromatin was more compact than that seen in myeloblasts, the cytoplasm was pale blue. There were transitional forms tending towards *the prolymphocytes with one nucleolus*, but otherwise a lymphocyte-like nucleus The cells varied in size, but showed greater variation in size than normal lymphocytes in the peripheral blood A moderate number of smear cells. Scanty mitoses of the lymphoid cells.

This case showed almost purely lymphoblastic marrow (Figs. 126–128), while in the peripheral blood, apart from primitive forms, there were also numerous senile forms. There was aplasia of *erythropoiesis and thrombopoiesis in the marrow. A* hæmorrhagic diathesis did not occur, but there was a progressive, hypoplastic anæmia. Granulocytopoiesis was also *suppressed*. When acute lymphatic leukæmia is localized in the marrow exclusively, it seems that the other marrow systems suffer just as in acute myeloid leukæmia.

Summary. Sternal puncture can often help in the diagnosis of acute *lymphatic leukæmia*, because the marrow appears to be affected by the lymphatic hyperplasia early in the disease.

MONOCYTIC LEUKÆMIA

This subject is full of controversy The problem has been extensively reviewed by Roversi and Salaris (1938), Schultz and Kruger (1939), Thaddea and Bakalos (1939), Watkins and Hall (1940), Hittmair (1942), and others. Reschad and Schilling (1913) were the first to describe the disease.

Owing to the diversity of opinion on the theory of cell development, monocytic leukæmia is not universally recognized as a proliferation of the third cellular system The dualists (Naegeli, 1931, Jagić and Klima, 1937, Rohr, 1940, Hittmair, 1942; Piechl, 1943, and others) who hold that the monocyte is derived from the myeloblast, regard monocytic leukæmia merely as an *atypical form of* myeloid leukæmia. American authors and Whitby and Britton (1946) recognize two types · (1) *myeloid monocytic leukæmia (type Naegeli)* and (2) reticular monocytic leukæmia (type Schilling). As far as *diagnosis* is concerned, Hittmair makes it a condition that at least 50% of the blood cells should be monocytic in both types. Cases of the Schilling type have been described by Ewald (1923) as leukæmic reticular endotheliosis, the case reported *by Swirtschewskaja* (1926) had up to 96% monoblasts and monocytes in the blood Bock and Wiede *(1930), Hittmair* (1942), Engback, Heerup and Thomsen (1942) have also described similar cases Watkins and

Hall (1940) collected 23 cases of the type Naegeli and 6 cases of the type Schilling. They describe as the stem cell for the first type the myeloblasts with coarse granulation, which develop successively into finely granular monocytes. According to their view the stem cell of the Schilling type is a large reticuloendothelial cell with eccentric, round or oval, furrowed nucleus and basophilic cytoplasm. The course of both types is similar. There are acute as well as chronic varieties, the duration in the Naegeli type varied between 5 weeks and 6 years, in the Schilling type between 7 weeks and 27 months. The number of leucocytes was between 2,700 and 720,000, and between 2,600 and 40,000 respectively.

Aleukæmic and leukæmic forms occur. Schilling, and Watkins and Hall consider that the type Naegeli is a form of myeloid leukæmia with many paraform cells. Thaddea and Bakalos (1939), on the other hand, on the basis of their observations, formulated a theory on cell development which has been discussed already. They completely divorce the neutrophil series from myeloblasts and promyelocytes and maintain that in the acute leukæmias, as well as in many instances of agranulocytosis, it is the promonocytic-monocytic system which is reacting.

It is questionable whether differentiation of the myeloid monocytic leukæmia from the parapromyelocytic-paramyeloblastic leukæmia is at all possible hæmatologically. The only point of difference is the absence of the myeloid hiatus leukæmicus, because the mature cells in monocytic leukæmia are alleged to be monocytes and not mature neutrophils. In Case 29 (p 215) of subacute myeloid leukæmia, we decided, in spite of the bizarre lobation of the cells, in favour of the diagnosis of paramyeloblastic leukæmia because there was an hiatus leukæmicus and involvement of all three granulocytic series. The classification of the monocytoses, which may be chronic and may be accompanied by skin manifestations (Schilling, 1938, Anagnostu, 1939) is possible clinically (splenomegaly, etc), and histologically. The cases of Krummel and Stodtmeister (1936), Whitby and Hynes (1936), Thaddea and Bakalos (1939), Hittmair (1942) and others belong to the myeloid monocytic leukæmias. Following Ewald (1923) the reticular monocytic leukæmia (type Schilling) is called leukæmic reticulo-endotheliosis by Hittmair, but Engbaek, Heerup and Thomsen (1942) do not like this term. Whereas in the type Naegeli the proliferation starts in the marrow and can always be observed there, in the type Schilling extramedullary proliferation predominates. Transitional and mixed forms occur, but they are mostly myeloid monocytic leukæmias with pronounced reticulo-endothelial reaction as well. According to Cioni (1938) the division into parenchymatous (leukæmia) and stroma (reticulosis) reaction is not justified, because leukæmia always leads to stroma reaction. The aleukæmic forms of reticular monocytic leukæmia correspond

to reticulo-endotheliosis (Ritchie and Meyer, 1936; Zolezzi, 1937; Engelbreth-Holm, 1938; Hittmair, 1942) in which is found histologically the same reticulum proliferation, but without extramedullary hæmopoiesis arising from *the cells of the reticulo-endothelial* system. Schultz and Kruger (1939) state that it is still not clear whether monocytic leukæmia is a disease of the reticulo-endothelial system or of the mesenchyme in the wider sense. Strict division into leukæmic and aleukæmic reticuloses is necessary according to Hittmair *to avoid an increase in the already prevailing* confusion.

Osgood (1937), Plum and Thomsen (1938), Thaddea and Bakalos (1939), Bock (1939), Hagio (1939), Falzoi (1939), Schultz (1940), Meyer and Flanagan (1941), Sweany and Cannemeyer (1941) found *monocytes and their precursors (monoblasts and promonocytes) in* the sternal marrow. Krummel and Stodtmeister (1936), de Weerdt (1939), Piechl (1943) and others found myeloblasts in myeloid monocytic leukæmia. In Krummel and Stodtmeister's case of a woman aged fifty-seven, besides myeloblasts there were also many *monocyte-like cells, which disappeared with radiotherapy, and* gave place to myeloblasts. After the reaction due to irradiation had subsided, the monocyte-like cells once more predominated. Thaddea states that in the terminal stages primitive and atypical myeloblasts from the marrow are released into the blood stream. *This, however, is not evidence for the transition* of monocytic leukæmia into acute myeloid leukæmia, because these primitive cells have been found in the marrow from the very onset of the disease. De Weerdt (1939), who in his 3 cases found a blood monocytosis between 57·3% and 84%, observed myeloblasts in the marrow, *and like Lambin (1937) takes the view that monocytic* leukæmia is not a reticulosis, but a myelosis with accompanying reticulosis in the sense already discussed.

Leukæmic abnormalities of the monocytic precursors have been described by Rinehart (1932), di Guglielmo (1937) and others; they include *irregular cell edges, pseudopodia-like cytoplasmic* buds, peripheral basophil reaction and perinuclear oxyphil reaction of the cytoplasm, syncytium-like nests and numerous mitoses in monocytes. Strangmann (1943) counted monocytes according to Arneth's method and found a shift to the left in the early stages, *and a shift to the right of the monocytes* in the later stages.

It is clear from these results, that the problem of monocytic leukæmia has not been settled by marrow biopsy. To illustrate the difficulty of diagnosing individual cases, we quote Strangmann's case, where marrow biopsy failed to classify the cells. Apart from *typical myeloid cells,* monocytoid forms were also present. At autopsy acute myeloid leukæmia was revealed with myeloblasts, promyelocytes, myelocytes, metamyelocytes and stem cells in bone marrow, promyelocytes and myelocytes in the spleen and myelocytes

in the liver. Although monocytes were present in the peripheral blood we believe that this was not a case of monocytic leukæmia in Schilling's sense, but a myeloid form. Schultz, in whose department Strangmann's work was done, insists, however, on the trialistic theory as far as leukæmia is concerned, and we share his view. He believes that monocytic leukæmia is of reticular origin. According to Arneth, a leukæmia can be called monocytic only, if at the same time the lymphocytes, which according to his theory give rise to the monocytes, show a leukæmic reaction. These considerations show clearly that the problem of leukæmia is largely complicated by the different theories of cell development.

We believe with Watkins and Hall, Hittmair and others, that the myeloid monocytic leukæmia (type Naegeli) is a variant of myeloid leukæmia. We recognize the leukæmic reticulo-endotheliosis (type Schilling) only as true monocytic leukæmia.

MEGAKARYOCYTIC LEUKÆMIA

Since Dubinskaja (1928), first described megakaryocytic leukæmia many authors have reported cases (Boros and Korényi, 1931; Hugonot and Solier, 1935; Weil, *et al*, 1936; Hewer, 1937; Dustin, 1938; Lindeboom, 1938; Matthaeus, 1938; Bamforth and Kendall, 1939; Downey and Nordland, 1939; Essbach, 1939; and Guttner, 1941). The separate entity of this disease, however, is not universally acknowledged. As early as 1904, Schwarz had observed megakaryocytes in the blood stream in myeloid leukæmia. Naegeli (1931) did not consider them unusual in this disease. Kaznelson (1916), Ricci (1922), Rettano (1922), Minot (1922) and Minot and Buckman (1923) made similar observations. This explains why megakaryocytic leukæmia has been regarded as a variation of myeloid leukæmia, in which megakaryocytes are transported from the marrow to the blood stream and to other organs. Whitby and Britton (1946) point out that megakaryocytes are found in the blood not only in myeloid leukæmia, but also in polycythæmia vera and especially in myelosclerosis and osteosclerosis. They do not believe megakaryocytic leukæmia is a separate entity. According to Matthaeus, megakaryocytes are found only in certain organs, especially liver and spleen, and the formation of megakaryocytes in these sites by metaplasia of the littoral cells appears to be more probable (Matthaeus, Guttner, Essbach). Lindeboom, and Downey and Nordland emphasize the strict topographic division of megakaryocytic and myeloid infiltrations in liver and spleen, a point in favour of the different nature of the two processes.

The bone marrow is not infiltrated uniformly, but in a focal manner. Bamforth and Kendall (1939) noted much mature myeloid tissue and many megakaryocytes. Dustin reported an active

marrow, poor in megakaryocytes. Most authors agree with Downey and *Nordland that megakaryocytic hyperplasia in liver and* spleen is characteristic, but lymph glands, kidneys, lungs, and other organs may show a metaplasia just as in myeloid leukæmia. Weil, *Chevallier and Sée (1933)*, Favre, Croizat and Guichard (1934), and Lindeboom (1938) have reported aleukæmic forms. Lindeboom states that it is a chronic disease of the middle years of life with *splenic and hepatic* enlargement and myeloid reaction in the blood. The marrow is affected only slightly at first, and later on more definitely. He classifies the disease between the leukæmias and endothelioses as a reticulo-endotheliosis, giving it the name "hepatolienal hæmatopoietic endotheliosis."

PLASMA CELL LEUKÆMIA AND MULTIPLE MYELOMATOSIS

While there is much controversy about the separate entity of monocytic leukæmia and even more so about megakaryocytic leukæmia, the existence of plasma cell leukæmia is recognized by most authors. Marrow biopsy has contributed much towards the knowledge that myelomata are proliferations of plasma cells, and has shown that the old-fashioned diagnoses of myelocytic and erythroblastic myeloma were due to the unfavourable working conditions which are encountered when the material for histology is obtained exclusively from the post-mortem room Aschoff (1906), Berblinger (1911) and others reported plasma cell myelomata, and younger generations of morbid anatomists, such as Apitz (1940), Brass (1943, 1944) follow them and regard myelomata as plasmocytomata. We have grouped plasma cell leukæmia and multiple myelomatosis (Kahler's disease) into one section, because the differences between the two conditions are those of degree only. Attempts have been made to differentiate between multiple myelomatosis and plasma cell leukæmia, but we do not believe that the basically common features can be disregarded Multiple myelomatosis as a localized, tumour-like proliferation is characterized by —

(1) Pains, and abnormal fragility of the bones.

(2) Progressive cachexia

(3) Bence-Jones' albuminuria (*Kahler's Triad*)

(4) Typical X-ray appearances of bones with multiple translucent areas (bone destruction).

(5) Hyperproteinæmia, or rather *paraproteinæmia*, with an increase in globulin

(6) Plasma cells in the sternal marrow

Amyloid disease *of the kidneys may* occur as reported by Askanazy (1927) and Magnus-Levy (1933) According to the well planned investigations by Brass (1943, 1944), these are actually cases of nephrosis, *following* the excretion of proteins which are

foreign to the blood. In plasma cell leukæmia the fragility of bones is usually absent, and so are Bence-Jones albuminuria, the typical X-ray picture of bone destruction and sometimes also hyperproteinæmia. Keilhack (1943), however, states that hyperglobulinæmia is constant and we have seen Bence-Jones albuminuria in a case of plasma cell leukæmia. The uric acid level in blood and urine is often raised, indicating increased blood cell destruction, and terminally a hæmorrhagic diathesis may occur just as in other leukæmias.

Pathologically the difference between the two states is not marked. Extramedullary foci of hæmopoiesis are found in multiple myelomatosis as well as in plasma cell leukæmia. Borst (1928) believes that extramedullary foci in the liver, spleen and other organs in multiple myelomatosis are not tumour metastases, but areas of extramedullary hæmopoiesis, just as in other leukæmias. Ribbert (1904) on the other hand, regarded the multiple foci of plasmocytoma in the marrow itself as metastases. Askanazy (1927) and Jaffé (1933) take an intermediate view and speak of colonization.

Askanazy and Dubois-Ferrière (1942) suggest the following classification :—

(1) Multiple myelomatosis with or without a terminal crisis consisting of a shower of plasma cells in the blood.

(2) Multiple myelomatosis with plasma cell leukæmia (Gluzinski and Reichenstein, 1906; Muller and McNaughton, 1932, Patek and Castle, 1936; Schilling and Wohlenberg, 1938; Steinmann, 1939, and others).

(3) Aleukæmic plasma cell leukæmia (case of Reiter and Freeman (1937) with 5,200 W B C, 49% being plasma cells).

(4) Plasma cell leukæmia (Piney, 1924, Osgood and Hunter, 1934, Fleischhacker and Klima, 1936, Lachnit and Walterskirchen, 1939, Keilhack and Linck, 1941)

(5) Plasma cell leukæmia with myeloma (case of Askanazy and Dubois-Ferrière (1942), in which only two small foci of plasmocytoma could be found in the spine).

Leitner, (1944) attributed the islet-like localization to the small number and the irregular distribution of plasma cells in the marrow Askanazy and Dubois-Ferrière emphasize the islet-like localization in the diagnosis of plasmocytoma, but Apitz (1940) and Brass (1943) point out that cases of diffuse plasmocytosis are not rare and do not essentially differ from plasmocytoma There are however, certain differences between the various forms of plasma cell proliferation The composition of the blood proteins in single myeloma differs from that in multiple myelomatosis even when the Takata-Ara reaction is negative. In plasma cell leukæmia as a rule there is no bone destruction. It is just possible that there may be some relationship between the type of blood proteins and the number and extent of the foci of plasmocytoma

Morphological hyperplasia, as we have pointed out before, is not necessarily accompanied by increased function.

Christian (1907) and Krjukof and Korovnikof (1928) showed that the myeloma cells belonged to the plasma cell group, and *Wallgren (1920) reported the uniform nature of the myeloma cells.* The findings of solid collections of plasma cells in sternal marrow by Zadek and *Lichtenstein (1931, 1932) and Henning (1938)* has been confirmed by Schulten (1936), Skouge (1936), Ferrata and Storti (1937), Curtze (1938), du Bois (1938), *Nielsen (1938), Schilling and* Wohlenberg (1938), Kuthan (1939), Packalén (1939), Steinmann (1939), Weisenbach and Lièvre (1939), Rettanni (1940), Keilhack and Linck (1941), Melle and Cornelis (1941), Askanazy and Dubois-Ferrière (1942), Magyar (1942), Waldenström (1942), Michaud (1943), Schupbach (1943), Jeanneney, Gounarin and Tingaud (1944), Jéquier-Doge and Chapuis (1944), Leitner (1944) and Pedrazzini (1944). *Negative findings have been reported by Duvoir, Layani,* Padovani and Laudat (1938), Curtze (1938), Schupbach (1943) and Leitner (1944), which is *quite comprehensible because sternal* marrow is usually though not invariably affected by plasma cell proliferation. In such cases sternal puncture should be performed at the site of election, i.e., where the bone is tender, or where there is an area of translucency on the X-ray photographs. In a case which gave a negative result on sternal puncture, we were able to establish the diagnosis by puncturing a rib which was tender on pressure. *In the early stages the myelomatous proliferation* is only slight.

cell marrow

Sirridge (194 ,

cases of plasma cell myeloma. They noted an increased number of plasma cells, plasmoblasts and plasma cells in mitosis; nuclear abnormalities in the plasma cells (multiple nuclei, nuclear fragments and indented and lobulated nuclei); *cytoplasmic abnormalities* (variation in size, vacuoles, a tendency to large forms and *irregularity in shape with cytoplasmic* projections); relative and absolute decrease in myeloid, erythroid and megakaryocytic elements with a tendency towards immaturity, a slight lymphocytosis They consider that the plasma cells arise from reticulum cells and constitute a distinct line of cells different from myeloid, lymphoid or erythroid types. *Lichtenstein and Jaffe (1947)* surveyed a series of 35 cases, and point out the comparative infrequency *of Bence-Jones' proteinuria and of multiple* osteolytic foci Most cases develop normocytic, hypochromic, progressive anæmia. Immature erythroid and myeloid cells appear in the blood and may produce a leukæmoid picture The erythrocytes form excessive rouleaux and may clump in Hayem's solution Atypical plasma or myeloma cells occur, and may form up *to 65% of all marrow cells.* There is a small cell type resembling plasma cells, and a larger, often

bizarre type with vacuolation and frequently with "Auer-rods," both inclusion bodies probably being of a protein nature. The ætiology and the production of the disordered protein metabolism is not yet fully understood.

We have examined 4 cases of multiple myelomatosis and 2 cases of plasma cell leukæmia by marrow biopsy. In 2 cases of the 4 we found numerous plasma cell groups, in one other we found none. The fourth case of myelomatosis showed 37.5% plasma cells in the first puncture, and 51.5% in the second. Diagnosis was established by the first puncture, because myeloid hyperplasia was absent, which distinguished it from plasmocytosis due to infection. This case was of interest also because of marked eosinophilia, and because many of the cells showed numerous large, atypical vacuolations, partly intracellular and partly extracellular, and because it enabled us to draw conclusions about the formation of proteins by the myeloma cells.

Case 31. B. H., aged 64, a head librarian. As a youth had had asthma. 1940 nervous complaints. Chest radiograph normal. Sedimentation rate (Westergren) 12/45 mm (1 and 2 hr). Hb. 95% = 15.2 g %. June, 1943, pneumonia, treated by Irgafen (dimethyl-benzoyl-sulphanilamide). Marked anæmia with 2.0 million red cells and Hb. 43% = 6.9 g %. Sedimentation rate 83 mm. The anæmia improved a little with liver and iron therapy and the patient's weight increased, but he continued to complain of pains in the chest and looked tired and ill. Sixteen blood counts were carried out during four months, but we include only the one with the most marked eosinophilia.

BLOOD. R.B.C. 4.18 millions, Hb. 78% = 12.5 g %, W.B.C. 7,250, basophils 0.5%, eosinophils 22%, stab forms 0.5%, segmented polymorphs 44%, lymphocytes 29%, monocytes 4%. Platelets 410,000, later 200,000. Sedimentation rate (Westergren) at the beginning 32/70, later 96/140 mm. Serum proteins 10.15 g %, albumen 2.84 g %, globulin 7.31 g %. Non-protein nitrogen 20.2 mg %, calcium 10 mg %, inorganic phosphorus 5.1 mg %. Takata-Ara ++. Weltmann reaction much narrowed, later broadened.

1ST STERNAL PUNCTURE (September, 1943). Proerythroblasts 2, early basophilic normoblasts 3.25, normoblasts 39.5 per 100 white cells, myeloblasts 0.25%, promyelocytes 1.75%, neutrophil semimature myelocytes 3%, mature myelocytes 5.25%, metamyelocytes 11.75%, stab forms 12.5%, segmented polymorphs 10.5%, eosinophil myelocytes 5%, eosinophil metamyelocytes 2%, eosinophils 2.25%, basophil myelocytes 1.25%, basophils 0.25%, lymphocytes 4.5%, monocytes 0.75%, megakaryocytes 1%, primitive reticulum cells 1%, phagocytic reticulum cells 0.25%, fat cells 0.25%, plasma cells 37.5%.

2ND STERNAL PUNCTURE (January, 1944). Proerythroblasts 1.25, early basophilic normoblasts 3, normoblasts 34.75 per 100 white cells, myeloblasts 0.25%, promyelocytes 1%, semimature myelocytes 0.5%, mature myelocytes 4%, metamyelocytes 6.25%, stab forms 5.25%, segmented polymorphs 11.25%, eosinophil myelocytes 5.5%, eosinophil metamyelocytes 3%, eosinophils 1.25%, basophil myelocytes 0.5%, lymphocytes 1%, monocytes 0.5%, megakaryocytes 1.5%, primitive reticulum cells 0.5%, phagocytic reticulum cells 0.5%, plasma cells 51.5%. A third sternal puncture was carried out after radiotherapy and showed marked

reduction of plasma cells in the sternal marrow. The blood protein picture, however, remained quite typical: serum proteins 11·5 g.% (Howe's method), with an increase of the euglobulin fraction.

Both sternal punctures showed the granulocytopoiesis to be

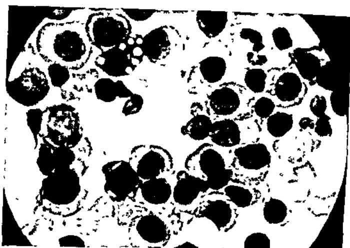


FIG 129 Marrow with plasma cells in multiple myelomatosis In the upper part of the field is a cell with large, bluish vacuoles, such cells were very numerous in this particular case. (×950)

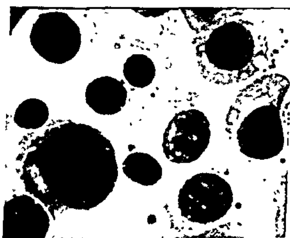
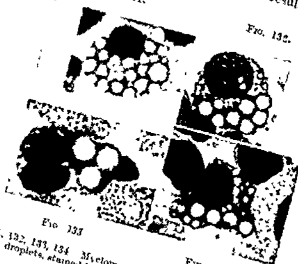


FIG. [illegible]

hypoplastic, thus excluding an infectious type of plasma cell reaction, quite apart from the fact that such a pronounced plasma cell increase has never been seen in infection. Erythropoiesis was slightly hyperplastic, which accounts for the improvement in the anæmia (Hb rose from 43% to 72%) The bone pains disappeared with radiotherapy. The diagnosis of "plasmocytoma" was established

by the first sternal puncture. Bence-Jones' proteinuria was absent. X-ray photographs of the skeleton showed general atrophy but without foci of translucency. Blood chemistry revealed marked hyperproteinæmia of 10·15 g %, with the high figure of 7·31 g.% for globulin, a strongly positive Takata-Ara reaction, and an initial narrowing and, later, broadening of Weltmann's coagulation band. The sternal puncture and the blood protein picture were typical for plasmocytoma. In the narrow there were large compact collections of plasma cells (Figs. 129, 130). The cytoplasm of the cells was often missing; there were many mitotic figures in plasma cells, and many multinucleate plasma cells, the result of atypical cell division.



Figs. 131, 132, 133, 134. Myeloma cells with abnormal, large plasma cell droplets, stained blue with varying intensity. (×1,850)

The large extracellular and intracellular vacuoles of the plasma cells were particularly noteworthy. They were, especially the large ones, often of a bluish colour and must be considered as a secretion of the plasma cells, globulin or rather paraglobulin by nature and not a type of vacuole, which as extracellular bodies would not have taken up the stain. The fact that they are protein bodies is indicated by the negative result of a stain with iodine for carbohydrates and their failure to stain with Sudan III. These structures are probably equivalent to the Russell-bodies of the morbid anatomists (Figs. 131–137).

The following case, published by Steinmann (1939), whose preparations I was able to examine, is especially remarkable for the enormous numbers of crystals resembling Auer-rods in the plasma cells. As these cells were present in peripheral blood, the case should be called plasma cell leukæmia.—

Case 32. N. S., a woman of 51 years, since the beginning of 1938 suffered from lassitude, weakness, loss of weight (about 10 kg), shortness of breath and thirst. Frequent attacks of diarrhœa, menorrhagia and anæmia. When examined she was pale, the spleen was just palpable, the heart was enlarged to the left, with a systolic murmur at the base. The sternum, at the level of the third and sixth ribs, and the right iliac crest were tender to pressure. X-rays: translucent areas in the neck of the femur, ischial tuberosity, pubic bone and left iliac bone. Bence-Jones' albuminuria 1·2 g.%. Urine showed a few casts

BLOOD. R B C. 2·4 millions, Hb. 56%=9 g.%, C.I 1·16; W.B C. 12,000; eosinophils 1%, myelocytes 0 5%, stab forms 0 5%, segmented polymorphs 24·5%, monocytes 3%, lymphocytes and lymphoid cells 40 5%, plasma cells 30%, of which 5 5% showed Auer-like rods and

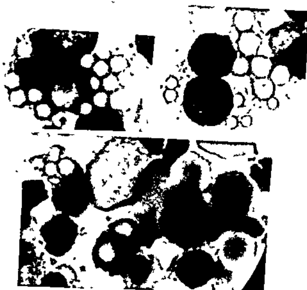


FIG 135. FIG. 136.

FIG 137

FIG. 135 Coalescing intracellular, dark blue plasma cell droplets
FIG 136 Bluish plasma cell droplets, some intracellular and some extracellular

FIG 137 Large, extracellular dark blue plasma cell droplets (×1,050)

7% showed Auer-granulation (Fig 138) Serum protein 6 04 g %, albumen 2·59 g %, globulin 3 45 g % (euglobulin = 1 53 g %, pseudoglobulin I = 1 07 g %, pseudoglobulin II = 0 85 g %) Cholesterol 74 mg.-%, Takata-Ara reaction negative

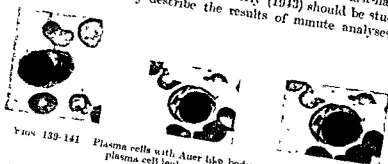
STERNAL MARROW. Almost entirely consisted of the plasma cell series, 94% of all cells were plasmoblasts, proplasmocytes or plasma cells, myelocytes 1%, metamyelocytes 1%, stab forms 1%, eosinophils 0 5%, megakaryocytes 0 5% Scanty erythroblasts, 1 per 100 white cells In histological sections of the marrow obtained by puncture, tumour tissue with round cells could be seen Progress was downhill anæmia increased (Hb 14% = 2 3 g %, R B C 600,000) Blood transfusions effected transient improvement only

This was a case of multiple myelomatosis with plasma cell leukæmia. The serum proteins were not increased, a fact which in the presence of marked albuminuria has been observed by many other authors (Freund and Magnus-Levy, 1932; Alder,



FIG 138 Sternal marrow in plasma cell leukæmia Group of plasma cells with Auer like bodies (×1,050)

1936; v. Bonsdorff, Groth and Packalén, 1938; Keilhack, 1943) The negative Takata-Ara reaction may be explained similarly. A positive reaction, very frequently observed by Gros (1935) in plasmocytoma, depends on the increase of euglobulin and pseudo-globulin, and probably also on some other fibrinogen-like body (Wuhrmann and Leuthardt, 1938) The papers of the last-named authors and of Wuhrmann and Wunderly (1943) should be studied in the original as they describe the results of minute analyses of



FIGS 139-141 Plasma cells with Auer like bodies in peripheral blood in plasma cell leukæmia (×1 050)

protein bodies carried out by Butler and Montgomery's fractional precipitation method

This case unlike Case 31, is remarkable for the coarse, often rhomboid Auer-rod-like structures We agree with Steinmann that these are protein bodies (Figs 138–141), and represent the precipitated crystalloid form, while the coarse, atypical vacuoles (better named plasma cell droplets), which were also present, represent the liquid form of the abnormal protein bodies Both cases of multiple myelomatosis sh— well the part played by

myeloma cells in protein formation. The morphological abnormality probably corresponds to a structurally atypical form of protein ("paraprotein"). The theory of blood protein formation has already been discussed extensively (pp. 43–48). Recently Snapper and Schreid (1946) and Snapper (1947) have found that the administration of Stilbamidine, or Pentamidine, combined with a low protein diet had a favourable influence on the pain associated with multiple myeloma, although it did not cure the disease. These drugs induce morphological changes in the myeloma cells as seen in sternal puncture. *Large basophilic granules appear in the* cytoplasm and show a tendency to become confluent. They appear to be precipitates of ribonucleic acid.

Summary. (1) Sternal puncture is valuable in the diagnosis of plasma cell myeloma; it often decides the diagnosis. (2) With certain reservations it can be used in assessing the prognosis. A slight infiltration with plasma cells is often seen in the early stages. (3) Apitz *believes that it may aid in deciding on suitable therapy.* Circumscribed myelomata without generalization throughout the body are alleged to be operable. (4) Sternal puncture observations have contributed *significantly* to the knowledge of multiple myelomatosis. (4) They have produced valuable insight into the protein-forming part played by myeloma cells and by plasma cells. (6) The relationship between multiple myelomatosis and plasma cell leukæmia with all intermediate types is, in our *opinion*, an important argument in favour of the tumour-like nature of the leukæmias.

LEUKÆMOID REACTIONS

Those who believe in the infectious origin of leukæmia do not recognize leukæmoid reactions. They regard all cases showing a leukæmoid blood picture as leukæmias. Many others have attempted differentiation :—

(1) Pathologically the leukæmoid reaction is characterized by the continued existence of the normal anatomy of the marrow space [illegible] shift to the left, which is dis- [illegible]feration of the leukæmic [illegible]

(2) A favourable end result and good progress indicate a leukæmoid reaction

(3) Hæmatologically the morphology of mature and immature cells in leukæmoid reaction is said to remain normal (*Hill and Duncan*, 1941). Paramyeloblasts and the hiatus leukæmicus do not occur in it (Reinwein and Rösing, 1938; and *others*).

We have, however, pointed out that in leukæmoid reactions the morphology is often not normal, and that paraforms with all the

stigmata of paramyeloblasts may occur. On the whole in most cases diagnosis is possible hæmatologically, especially as a considerable myeloblastosis and the hiatus leukæmicus do not occur in leukæmoid reaction.

Rietti (1917), Bonnamour and Chapuis (1921), Baar (1924), Marzullo and de Veer (1931), Custer and Crocker (1932), Leitner (1938), Reinwein and Rösing (1938), Sterner (1939), Stöger (1940), Braun (1943) have reported leukæmoid reactions in tuberculosis; Krummel and Stodtmeister (1936) in paratyphoid B septicæmia; Krumbhaar (1926), Osvaldella (1932), Leibowitz (1938), Brustlein (1939), and Heck and Hall (1939) in septicæmia; Hill and Duncan (1941) in osteomyelitis and in Hodgkin's disease; and Erkelentz (1935), Muller (1938), Forconi and Carere-Comes (1940), Hill and Duncan (1941), Leitner (1945) and others in malignant disease and Britton and Warner (1945) in phlegmonous gastritis. Downey, Mayor and Noble (1930) record myeloid blood pictures in mercury poisoning and Krumbhaar (1926) in mustard gas poisoning, sulphadiazine (Whittemore and Stick, 1942), sulphapyridine and sulphathiazole (Kracke, 1944) have also been implicated Parrisius and Heimberger (1924) reported cases due to bee stings, one of the patients had a count of 120,000 leucocytes per cmm. with 69% of primitive myeloid cells. As far as tuberculosis is concerned, we have reviewed the relevant literature (Leitner, 1935), and come to the following conclusions:—

(1) In many cases (Quincke, 1902, Hemmerling and Schleussing, 1927; Holler, 1931; Lenhartz, 1932; Jaffé, 1933; Weber and Schluter, 1936, and Case 29) tuberculosis and myeloid leukæmia occurred together, though ætiologically completely independent of each other Junger (1900), Weinreich (1931), Fischer (1935), Ryan and Medlar (1937) similarly described tuberculosis and lymphatic leukæmia We do not believe tuberculosis to be an ætiological factor for leukæmia Lenhartz (1932), Jaffé (1933), Sandmann (1933), and others have shown that tuberculosis has no aggravating effect on leukæmia, while Rebitzer (1892), Thorsch (1896), Quincke (1902), Dock (1904), Schwarz (1904), Lehndorff and Zak (1907), Mönckeberg (1912), Lowy (1921), Holler (1931), Naegeli (1931) and others have recorded apparent improvement in the leukæmia with decrease in splenomegaly and in the number of leucocytes Holler attributes such events to inflammatory changes in the spleen Inflammatory sclerosis in the spleen may be of such extreme degree that it becomes impossible to discover any leukæmic changes at autopsy This in turn makes diagnosis more difficult and makes caution very necessary when assessing findings at autopsy. According to Rohr's theory the decrease of leucocytes following sclerosis in the spleen is quite plausible Cases without any leukæmic changes at autopsy, on the other hand, such as those of Kruckemeyer (1940), Nielsen (1942), Bichel (1942) and others, cannot be regarded

as true leukæmias, because extramedullary leukæmic changes can never be confined to the spleen alone.

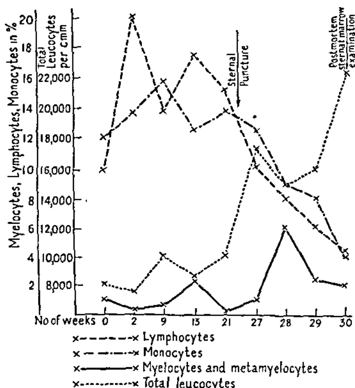
(2) Leukæmia on the other hand may play a rather larger part in the development of tuberculosis than *vice versa*. Ehrich (1934) and Leitner (1935) point out that only mature granulocytes are fully capable of defence against infection, and so, when primitive cells predominate, such defence may break down (Jaffé, 1933). This also explains secondary infections in leukæmia (Naegeli, 1931). When leukæmia co-exists, tuberculosis may often end with metastatic spread (Mönckeberg, 1912), or with rapidly spreading miliary tuberculosis (Hirschfeld and Tobias, 1902; Harbitz, 1936; Shen and Tajiri, 1935), or latent tuberculosis may become activated. Owing to the absence of defence, miliary spread does not lead to the formation of granulomata, but to areas of necrosis containing bacilli, i e., what Scholtz calls "sepsis tuberculosa acutissima" (Monckeberg, 1912; Krasso and Nothnagel, 1925; Eckel, 1929; Gosau, 1934, Wätjen, 1935; Leitner, 1935; Wuhrmann, 1941). Quite unjustifiably this condition has been identified with Landouzy's Typhobacillosis, which is a curable condition Tuberculosis does not necessarily become worse when co-existent with leukæmia as shown by Case 29 (p. 215), though it does when both processes are localized in the same organs

(3) Bonnamour and Chapuis (1921), Reinwein and Rösing (1938), Geissler and Wurm (1939) and Stöger (1940) have recorded leukæmoid reactions in tuberculosis. They appear to be especially frequent in a morbid state defined by us as "generalized caseating tuberculosis of the lymphatico-hæmopoietic systems" The cases of Engelbreth-Holm (1937), Reinwein and Rösing (1938), Geissler and Wurm (1939), Braun (1943), Patrassi and Torrini (1943) belong to this syndrome Though Braun, like Reinwein, speaks of "hepatolienal tuberculosis," caseous mesenteric and para-aortic lymph glands up to the size of a hen's egg were found in his case. Clinically this case was presumed to be one of acute leukæmia, in spite of the few myelocytes (9%) and the absence of myeloblasts in the blood picture and the slight shift to the left in the sternal puncture

Case 33. L R, a saddler of 28, fell ill with pleurisy, cough and sputum in June, 1937 When examined in May, 1938, he had marked swelling of the cervical, supraclavicular, axillary, inguinal and abdominal lymph glands The liver and spleen were enlarged Productive bilateral pulmonary tuberculosis of both upper and middle zones; sputum T.B positive Pyrexia up to 100° F Biopsy of a gland showed caseating tuberculosis

Blood R.B C 4·3 millions, Hb 81% = 13 g % W.B C 8,100, myelocytes 1%, stab forms 10%, segmented polymorphs 67%, lymphc [illegible] Graph 14 [illegible] platelets 336, [illegible] lood urea 17 mg %, cholesterol 156 mg %; protein 8 38 g %, albumen-globulin ratio 50 50, Takata-Ara reaction, negative

1st Sternal Puncture. [illegible] myeloblasts 0 5%, promyelocytes [illegible] mature myelocytes 5%, metamyeloc[illegible]mented polymorphs 54 5%, eosinop[illegible] 10%, promonocytes 4%, megakar[illegible]gocytic reticulum cells 0 5%. The patient's condition deteriorated steadily. He had high fever, the large glands softened and broke down Finally he succumbed to peritonitis, following erosion of the gut by caseous abdominal lymph gland masses Autopsy confirmed generalized caseous tuberculosis of the lymphatic system and the spleen

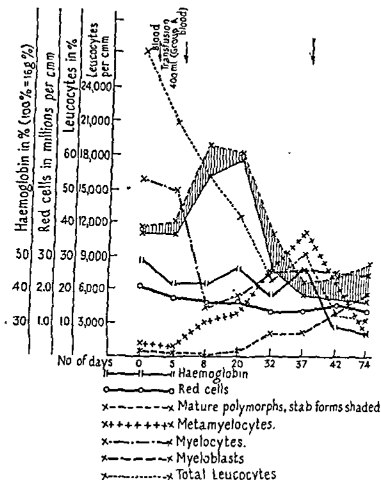


Graph 14 Leukæmoid reaction in generalized caseating tuberculosis of the lymphatic and hæmopoietic systems

2nd Sternal Puncture Immediately after death considerable increase of myelocytes, myeloblasts scanty as before, hypoplasia of erythropoiesis, normal figures of megakaryocytes

This was a case of "generalized caseating tuberculosis of the lymphatic and hæmopoietic systems," which we have defined as an atypical systemic tuberculous disease. Apart from a shift to the left and lymphopenia, the blood picture showed no abnormalities at the beginning; but terminally a leukæmoid reaction occurred with 5% myelocytes and metamyelocytes and with anæmia (R B C. 3 7 millions, Hb 70 2% = 11 3 g %) The high figure of 913,000

platelets which developed during the illness without an increase of megakaryocytes in the marrow was interesting, all the more as during the last few weeks of life the patient had an increased tendency to bleed (epistaxis). Bleeding time (Duke) was normal (1 min. in September, 2 min 30 sec. in December) and clotting time



GRAPH 15 Leukæmoid reaction in generalized caseating tuberculosis of the lymphatic and hæmopoietic systems with atypical nuclear division and promyelocytic marrow with lobed and vacuolated promyelocytes and myelocytes in blood and sternal marrow

(Burker) was not appreciably prolonged (11 min 30 sec in September, 4 min, 45 sec in December). We therefore suspected lack of Vitamin K from tuberculosis of the liver The first sternal puncture was all but normal The second, obtained only after death, showed a definite increase of myelocytes with toxic changes, thus excluding leukæmia. Autopsy findings do not justify the classification of this case as "hepatolienal tuberculosis" because caseous tuberculosis of the lymph glands predominated In the following case the leukæmoid reaction was even more pronounced

Case 34. Z. F., a pedlar aged 38 years, complained of abdominal pain during the last few years. In December, 1938, during some forestry work he injured his right hand, which began to suppurate. He was admitted to the dermatological clinic of the University of Berne, and then referred to our clinic with fever up to 101° F., a cushion-like swelling of the right hand, sedimentation rate (Westergren) 155–158 mm. (1 and 2 hr.) When examined his skin was dry, tongue coated, right axillary and submandibular lymph glands the size of hazel nuts. Lungs normal, heart enlarged, with an aortic systolic murmur; blood pressure 90/50. The liver edge was four fingers breadths below the costal margin. The spleen was not palpable with certainty, but enlarged as judged by percussion.

TABLE 12

Sternal Marrow Findings in Case 34

Cell types	Normal values	Case 34	
		30 1	6 3
Procrythroblasts } per 100 white cells	0-8	—	—
Early normoblasts } per 100 white cells	3 2	0 3	—
Normoblasts } per 100 white cells	24 4	5 3	3 0
Myeloblasts %	1 2	0 3	29 3
Promyelocytes %	2 2	10 3	24 4
Neutrophil semimature myelocytes %	5 4	20 0	7 3
„ mature myelocytes %	7 2	24 0	3 6
„ metamyelocytes %	10 2	11 6	3 3
„ stab forms %	24 0	9 0	8 3
„ segmented polymorphs %	28 4	10 0	8 3
Eosinophil myelocytes %	1 4	1 6	0 6
„ metamyelocytes %	0 8	0 6	—
„ polymorphs %	1 8	0 3	0 3
Basophil cells %	0 04	—	0 3
Lymphocytes %	8 6	6 0	5 6
Monocytes %	1 4	0 6	5 6
Megakaryocytes %	0 8	—	—
Plasmoblasts and plasma cells %	1 0	2 0	1 6
Lymphoid reticulum cells %	1 0	1 3	3 3
Phagocytic reticulum cells %	0 2	1 6	2 3

The blood pictures are shown in Graph 15. A leukæmoid reaction developed with 27,000 white cells, 52% of which were myelocytes, and 19% myeloblasts There was also marked anæmia with anisocytosis, poikilocytosis, Howell-Jolly bodies, 1% normoblasts, and after February 23rd, punctate basophilia. Anæmia increased in spite of transfusions After February 24th there was thrombocytopenia with prolonged bleeding time (16 min. 55 sec, Duke), and clotting time 15 min 25 sec (Burker)) Serum · Takata-Ara reaction, strongly positive, calcium 8 6mg %, bilirubin direct ++, indirect 2·8 mg % Two sternal punctures were made and the results are shown in Table 12. The patient died on March 7th, 1939 Autopsy



Fig 142 Immature myelocytic marrow in leukæmoid reaction with toxic granulation and vacuolation (× 500)

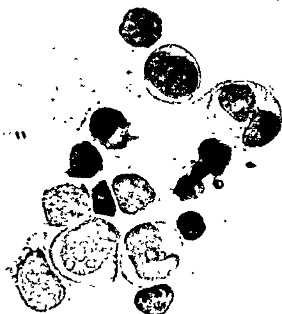


FIG. 143 Promyelocytic marrow with marked tendency to lobation in leukæmoid reaction (× 1,000)



FIG 144 Promyelocytic marrow with marked tendency to lobation and vacuolation of nucleus and cytoplasm in leukæmoid reaction (× 1,000)

(Institute of Pathology at the University of Berne) revealed a large spleen (19 × 12 × 5 cm.) with many caseous foci. Liver enlarged with whitish foci superficially and on the cut surface. Caseous cervical glands; bone marrow in the diaphysis of the femur brownish, and dark brown in the vertebræ.



Fig. 145.

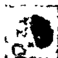


Fig. 146.



Fig. 147.

Fig. 145. Myelocytic marrow with abnormal nuclear division (binuclear nucleus and toxic granulation) in leukæmoid reaction (× 500)

Fig. 146. Myelocyte with marked vacuolation in leukæmoid reaction (× 500)

Fig. 147. Binuclear myelocytes with vacuolation in leukæmoid reaction (× 500)

There was thus a progressive myeloid reaction and aplasia of erythropoiesis and thrombocytopoiesis. In the first sternal puncture many abnormalities were seen. Myelocytes, promyelocytes and myeloblasts showed abnormal cell divisions, multipolar karyokineses, dissociation of paramyeloblasts and parapromyelocytes with a tendency to bizarre nuclear lobation and marked vacuolation of the cytoplasm (Figs. 143–151).



Fig. 148.



Fig. 149.



Fig. 150.

Fig. 148. Myelocyte with pronounced toxic granulation in leukæmoid reaction (× 500)

Fig. 149. Trinuclear myelocyte in leukæmoid reaction (× 500)

Fig. 150. Abnormal nuclear division, dissociation of nuclear and cytoplasmic division (× 500)

In this case, in which myeloid leukæmia was suspected, sternal punctures were of quite exceptional value in the establishment of the diagnosis. The marked toxic changes, such as abundant abnormal cell divisions, with multipolar karyokineses, and dissociation of nuclear and cytoplasmic division, producing myelocytes with three or four nuclei by suppression of division of the cytoplasm, extreme vacuolation, toxic granulation and karyorrhexis, indicated a disturbance of maturation, unusual in leukæmia. We have seen

binuclear myelocytes in myeloid leukæmia, but never in such abundance, and then without such marked vacuolation and without toxic granulation. In the second sternal puncture the similarity to a paramyeloblastic leukæmia was even greater, but we (Leitner, 1939) have pointed out that paraforms are not characteristic of leukæmia only. A hiatus leukæmicus was initially well marked in the blood picture as there were 53·5% myelocytes, promyelocytes and myeloblasts, the latter being 1% only, and 36% segmented polymorphs, the number of metamyelocytes being only 3·5% and stab forms only 1·5%. The hiatus disappeared later, and terminally there were 19% myeloblasts, 24% promyelocytes and myelocytes, 7% metamyelocytes, 12% stab forms and 16% segmented polymorphs. At the beginning differentiation would have been impossible, based on the blood picture alone, but the hiatus leukæmicus was missing from the sternal marrow throughout. The concurrent progressive anæmia with hypoplasia of erythropoiesis and of thrombocytopoiesis (73,000 platelets in February and terminally practically no platelets) and the absence of megakaryocytes, from the sternal marrow would have favoured a diagnosis of leukæmia very strongly, but this could not be verified at autopsy



FIG. 151. Abnormal nuclear division of an eosinophil in leukæmoid reaction. (× 1,400)

The difficulty of coming to a decision in such borderline cases is well illustrated by the post-mortem findings. We found caseating tuberculosis of the cervical lymph glands and the spleen, and miliary tuberculosis of lungs and liver. Smears taken from the marrow at autopsy showed many myeloblasts, but few myelocytes and polymorphs Sections of marrow from the femur showed many large round cells with basophilic cytoplasm and myelocytes, polymorphs, a few normoblasts and scanty megakaryocytes with pyknotic nuclei A moderate number of tubercle bacilli were seen in smears taken from the splenic pulp. Professor Wegelin, director of the Institute of Pathology at Berne, expressed the opinion that the diagnosis of leukæmia was doubtful There was no evidence of extramedullary hæmopoiesis It is quite possible that terminal tuberculosis produces a change in the picture of acute myeloid leukæmia, but as far as this case was concerned the absence of extramedullary hæmopoiesis and of the hiatus leukæmicus demonstrated by histological sections make the diagnosis of acute myeloid leukæmia unlikely and favour one of leukæmoid reaction

Summing up, the clinical and hæmatological as well as pathological and histological findings make it improbable that this was a case of acute myeloid leukæmia, but justify the presumption of a

myeloblastic reaction in the sense expressed by Stodtmeister. The name leukæmoid reaction, we believe, reflects the actual facts rather better than Stodtmeister's term.

In lymphatic reactions, e.g., in measles, whooping cough and chicken pox, sternal puncture usually makes differential diagnosis from lymphatic leukæmia possible, because lymphocytosis is absent in the marrow (Nordenson, 1935; Leitner, 1941). Smith (1941), Duncan (1943) and Steigman (1946) have described cases of infectious lymphocytosis following on upper respiratory infections, with an increase of lymphocytes in the sternal marrow up to 86% in Duncan's case, but these results were not confirmed by Lorenz *et al* (1946) or ourselves. Some of these cases may have been virus pneumonias, which, according to Moeschlin (1943) and in agreement with our own observations, may be accompanied by lymphocytosis.

Summary. Leukæmoid reactions occur in infections and in malignant disease. The diagnosis is often impossible on the basis of the blood picture alone. Sternal puncture, and more especially observation of the qualitative marrow picture, make differential diagnosis easier. Terminal infections may change the morbid anatomical picture of leukæmia, and in doubtful cases the diagnosis depends on post-mortem appearances and histological findings. The diagnosis of leukæmia is only justified in the presence of typical leukæmic changes in the organs.

AGRANULOCYTIC ANGINA (AGRANULOCYTOSIS, MALIGNANT NEUTROPENIA)

Sternal puncture is of great value in the diagnosis of agranulocytosis and has advanced the knowledge of its pathology. Most authors hold that agranulocytosis is a disease *sui generis*, but we believe that there is some sort of relationship to other morbid states, and that there are many transitions between agranulocytosis as a disease of a single cell system, aleukia hæmorrhagica (Frank) as an affection of two systems, and panmyelophthisic anæmia, as a disease of three marrow systems.

Agranulocytosis shows some predilection for certain age groups (according to Plum, 1937, it attacks patients chiefly between the ages of forty-five and sixty-three years) but it has also been observed in adolescents and even in children (Schmerel, 1934, Plum, 1937, Reznikoff, 1938; Glanzmann, 1942, Tobler and Buser-Pluss, 1942). Idiopathic and symptomatic forms of agranulocytosis are distinguished, but it is possible that with the progress made in diagnosis many idiopathic cases will be recognized as secondary forms. The term agranulocytosis, strictly speaking, can be applied only when the granulocytes have complety disappeared. Therefore, Naegeli (1931) proposed the name "granulocytopenia," and Schilling (1938) suggested the term "malignant neutropenia."

Though the latter term often reflects the actual blood findings more accurately, as exemplified by Case 36, the eosinophils and basophils being unaffected, we continue to use the name agranulocytosis, coined by *Schultz (1922), its discoverer. Rohr (1936) divides* agranulocytosis into three groups according to the course the disease takes

Acute Agranulocytosis

These cases show the typical symptomatology, described by Schultz; a general toxicity, often slight jaundice, tendency to necroses of the mucous membranes and slight angina ("agranulocytic angina"). Angina and jaundice do not invariably become manifest, but the decrease of leucocytes (granulocytes) is always definite and considerable. In the hyperacute form, which Nyfeldt (1939) calls "paroxysmal granulocytopenia," and which occurs suddenly while health is not impaired, the granulocytes may disappear completely from the blood within a few hours. Acute agranulocytosis is often attributed to some anaphylaxis in which drugs play a large ætiological part.

Plum (1937) in Denmark has observed parallelism between the ... *The experi-* ... isdorff (1936), ... lstorf (1938) and Lasch *(1940) have proved that amidopyrine can actually* cause agranulocytosis. They observed a prompt and short-lived decrease of granulocytes after minimal doses of amidopyrine, given to patients who had remitted from agranulocytosis apparently caused by this drug It has thus been possible to produce paroxysmal agranulocytosis experimentally in man. Since agranulocytosis following amidopyrine was discovered by Madison and Squier *(1934)*, numerous similar observations have been recorded by Groen and Geldermann (1934), Jackson (1934), Baeyer (1936), Dameshek and Colmes (1936), Grut (1937), Roch, Naville and Jendt (1937), Plum (1937), *Kahlstorf (1938), Lemierre, Laporte and Dépaillat* (1938), Seggel (1938) and others Dameshek and Colmes believe that amidopyrine produces anaphylaxis when coupled with body protein The antibodies, however, have so far not been isolated.

granulocytopenia in many tests in normal man with amidopyrine, while in animal experiments only very large doses of amidopyrine, such as are never used in human therapeutics, led to an agranulocytosis-like state (Butt, Hoffmann and Soll, 1939) We therefore, agree with Frank (1915), Naegeli (1931), Hoff (1934), Bock (1935) and Matthes (1937) that constitutional elements play a large part in the development of agranulocytosis. This view is also favoured by observations on the familial occurrence of agranulo-

cytosis, as reported by Aubertin, Blancstein and Lehmann (1929), Doxiades (1932), Zinninger (1934), Reynaud *et al.* (1938) and Huber (1939) The chief factor, however, appears to be sensitization, which is specific for every substance. Amidopyrine should not be prescribed during agranulocytosis, though it has been given with impunity by Limarzi and Murphy (1935) and Marcus (1935). It is impossible to ascertain in advance whether a patient is not already sensitized against amidopyrine, or whether he will become sensitized during the course of treatment. It is possible also that the allergy, just as in asthma, may become polyvalent as time goes by.

Neosalvarsan may lead to an acute but more frequently to a slow type of agranulocytosis (Bamforth and Elkington, 1931; Groen and Geldermann, 1934; de Jong, 1934, Bock, 1935; Colarizi, 1935; Mazzoleni and Sansone, 1937; Moeschl, 1939; Heintzelmann, 1940; Ferguson, 1944).

Following the administration of gold preparations, agranulocytosis has been reported by Jacob and Douady (1930), Achard *et al.* (1932), Wechsler(1937), Sondergaard (1939), Wintrobe, Stowell and Roll (1940) During systematic examinations of patients with tuberculosis and with rheumatoid arthritis, who were under treatment with gold, we (Leitner, 1938) have never encountered a case of granulocytopenia. In animal experiments, although gold was seen deposited in the bone marrow, we have failed to produce agranulocytosis, and we therefore believe that similar sensitizing conditions are at work with gold as in the case of amidopyrine.

At the present time interest is centred on the sulphonamides, which Glanzmann (1942) alleges play a more important part in the production of agranulocytosis than amidopyrine. Many cases of agranulocytosis from sulphonamides have been described, such as by Borst (1937), Berg *et al.* (1938), Bresgen (1938), Myhre (1938), Dolgopol and Hobart (1939), Sailer (1939), Selén (1939), Shecket and Price (1939), Sjöholm (1939), Tzanck, Arnous and Paillas (1939), Kennedy and Finland (1941), Lindeboom (1941), Bang (1942), Glanzmann (1942), Bickel and Dubois-Ferrière (1943), Kracke (1944) and Park (1944). Some of the cases were very severe; of Stjernberg's (1942) 10 cases, 7 died of agranulocytosis caused by sulphonamides Britton and Howkins (1938) have shown that sulphonamide drugs cause some reduction in leucocytes even in normal persons Bickel and Dubois-Ferrière have analysed the cases recorded, with special regard to sulphathiazole. With doses of 0 5 g and even 0 3 g. of sulphapyridine they were able to produce recurrences, which suggests some allergic process. The various preparations differ widely in their ability to produce agranulocytosis While sulphapyridine may lead to agranulocytosis rather frequently, sulphathiazole or Irgamid (N-1-dimethyl acroylsulphanilamide) do so very rarely. Cases due to sulphapyridine (Pringle *et al.*, 1940, Baker and Fenner, 1943), sulphadiazine (Blue,

1944), sulphaguanidine (Grant, 1944), sulphamerazine (Favorite *et al.*, 1944) and succinyl-sulphathiazole (Johnson, 1943) have all been reported. However, Huriez and Dumont (1942) gave a course of 90 g of sulphanilamide to a case of agranulocytosis for therapeutic reasons and attribute the cure to this drug, in conjunction with blood transfusion. Dameshek and Wolfson (1942) and Nixon *et al.* (1943) have also used sulphonamides in treatment with success. With the sulphonamides as with amidopyrine *caution is needed*, because it may be that hypersensitivity is specific for the sensitizing drug concerned. Because most drugs which may lead to agranulocytosis (amidopyrine, salvarsan, etc.) contain a benzol ring, Kracke (1938) deduces that the disease is actually caused by products of oxidation of benzol.

Many other drugs may cause agranulocytosis—for example, dinitrophenol (Goldman and Haber, 1936), thiourea and thiouracil (Haber, 1944; Limarzi and Ricewasser, 1946), bismuth (Dowds, 1937). An excellent review is given by Plum (1937).

Chronic Agranulocytosis

This form is frequently caused by infectious diseases: osteomyelitis, syphilis, chronic polyarthritis (Edström, 1941), frank septicæmia, malaria, abortus fever, typhoid, measles, mumps (Illing, 1939), kala-azar (Zia and Forkner, 1934), dibothriocephalus infestation (Schultz, 1942), spotted fever (Hoff, 1943) and tuberculosis. Experimentally, Wallbach (1932) produced agranulocytosis by injections of *B. coli* suspensions. Infections were soon incriminated as the cause of agranulocytosis (Kaznelson, 1916; Schultz, 1922, Friedemann, 1928). Tamalet (1941) and also Schur (1934) reported agranulocytosis in typhoid fever, and even following anti-typhoid inoculations, but Schultz believed that these cases cannot stand critical review, and he considered that anti-paratyphoid inoculation has never caused agranulocytosis In the chronic forms of agranulocytosis, in contradistinction to the acute allergic forms, splenomegaly usually develops. How far inhibition of the marrow is due to a splenic toxin is uncertain *Frequently* cures have followed on splenectomy, such as in the cases described by Hegler and Griesbach, (1931), Reissmann (1938) Nordenson and Rödén (1941) In addition Moore and Bierbaum (1939), Muether *et al.* (1941) and Wiseman and Doan (1942) describe a distinctive type "primary splenic neutropenia" which reacts well to splenectomy and which appears to be due to some *toxin formed by the spleen*

Marrow damage produced by toxic agents, such as Röntgen-rays, radium, benzol and others, also belong to the group of chronic agranulocytosis. Drugs which may cause reactions of hypersensitivity in certain cases may lead to agranulocytosis by toxicity

after prolonged administration. These include gold, salvarsan and others, and usually cause only a focal type of marrow damage without splenomegaly.

Symptomatic Agranulocytosis

Agranulocytosis in the course of some other marrow disorder has already been discussed in the chapter on the leukæmias. It may also occur in the reticuloses, Hodgkin's disease, etc., and should properly be separated from true agranulocytosis.

The marrow findings. The older reports based on autopsy material were very contradictory. Schultz and Versé (1922), Friedemann (1928), Barta and Erös (1929), Naegeli (1931), Wolff (1931), Jaffé (1933), Lovett (1934) and v. Bonsdorff (1937) found an aplastic marrow; Benecke (1917), Zadek (1925), FitzHugh and Krumbhaar (1932), Roche *et al* (1934), Turk (1938) and Braun (1944) found, however, normal or hyperplastic marrows. Cohen (1933) and Whitby and Britton (1946) distinguished therefore between plastic or maturation and aplastic forms. These contradictions have been cleared up to a large degree by marrow biopsy, especially by Rosenthal (1934) and Rohr (1936, 1939). Rohr's classification is the one most widely accepted He distinguishes the following types.—

(1) Normal or almost normal marrow, in which case agranulocytosis depends on a disturbance of the release mechanism of cells

(2) Maturation arrest with a promyelocytic-myelocytic marrow, in which cells ready for release into the blood stream are absent

(3) Aplasia of the granulocytic system with proliferation of marrow reticulum

(4) Aplasia of the granulocytic system with aplasia of marrow reticulum (fibrous, empty marrow).

(5) Agranulocytosis of peripheral type, depending on destruction of leucocytes (leucocytolysis).

Rohr's observations have been confirmed by many authors, amongst them Nordenson (1936), Markoff (1936), Klima (1938), Vischer (1938), Thaddea and Bakalos (1939), Leitner (1941), Kienle (1943), Limarzi and Ricewasser (1946) and others Fieschi (1936) considers that Rohr's classification is incomplete, as it does not include cases with a quantitative decrease of myelopoiesis, in which all stages of maturation are preserved The following case illustrates this —

Case 35. E A, a woman of 35 years, suffered with pressure headaches and disturbances of sensation in the hands, so that she had to give up her work, which involved the holding of thin metal wires in the production of telephone machinery.

BLOOD R B C 4 57 millions, Hb 84% = 13 5 g.%; W.B C. 4,150; basophils 1%, eosinophils 4%, neutrophils 39%, lymphocytes and monocytes 56%.

STERNAL MARROW. 2·25, normoblasts 18· promyelocytes 2·5%, se 14·25%, polymorph metamyelo 3%, megal proplasmoc %, lymphoid and phagocytic reticulum cells each 5%; total nucleated cells 28,880 per cmm.

This was a case of granulocytopenia, probably with a toxic basis, whose sternal marrow showed merely a slight myelocytic-metamyelocytic shift to the left. The total number of nucleated cells was reduced. Rohr (1936), Henning (1938), Thaddea (1941) and others have observed a slight shift to the left in similar cases. Like FitzHugh (1937), Rohr, Henning and others, we presume that the disturbance is not only one of the release mechanism in the marrow, but also one of maturation at least to a certain degree. Case 36 (p. 249) shows that the disturbance of the release mechanism may play an important part. Tobler and Buser-Pluss (1942), who worked out this case, failed to find evidence of increased peripheral destruction of the leucocytes, so that on the basis of marrow and blood findings the existence of a disturbance of the release mechanism must be considered to be proved. In cases with these slight degrees of disturbance of maturation and of the release mechanism a good prognosis can usually be given. Heilmeyer (1942) very wisely points out that extreme caution is necessary in giving a prognosis on the basis of marrow findings. In our experience this does not apply so much to type 1 cases as to the types with hyperplastic, immature or with aplastic marrow.

In agranulocytosis with considerable disturbance of maturation, the marrow is promyelocytic according to Rohr. Nordenson, Klima and others found that myeloblasts are not affected in agranulocytosis. Others record increases of myeloblasts in the marrow, especially in unfavourable cases, e.g., Custer (1935) in 9 of his 11 cases, Darling, Parker and Jackson (1936), Waitz and Hoerner (1938), Bruins Slot (1941), Kimura and Kumagai (1941). These findings cannot be due to a different interpretation of the myeloblast (possibly in Ferrata's sense), especially as, for example, Henning (1938) found 54% myeloblasts in one case. Naegeli (1931), Sabrazès and Saric (1935), Rohr (1939) found a short-lived myeloblastic reaction in cases of agranulocytosis which were beginning to recover. In such cases the differential diagnosis from leukæmia may be difficult, especially where immature cells circulate in the blood. Jackson *et al.* (1931), Strumia (1934), Rosenthal (1934) and Jackson (1934), met this difficulty, which we can confirm, especially when a large spleen is also present.

When the marrow is promyelocytic, monocytosis in the peripheral blood is often seen, and Lichtenstein (1932), Rosenthal (1934),

Strasser (1934), Bock (1937), Reznikoff (1938), Rohr (1940) and Whitby and Britton (1946) regard this as a favourable sign.

The course of the marrow reaction may be studied by repeated marrow biopsies. At the height of agranulocytosis Rohr found an immature myelocytic marrow. Four days later maturation set in once more, and eleven days later the marrow had become normal. Blood and marrow reactions did not proceed on parallel lines. While the number of mature cells in the marrow was still low, improvement could already be observed in the blood This was due to a rapid dispatch of the mature cells. The monocytosis in the blood, associated with a promyelocytic marrow is regarded as evidence for the myeloid origin of monocytes by Naegeli and Rohr. Thaddea and Bakalos (1939), on the other hand, speak of monoblastic-promonocytic marrow and, when a monocytosis occurs at the same time in the blood, regard it as proof of the independence of the promyelocytic-monocytic series of their terminology from the neutrophil series.. According to Thaddea, a promonocytic reaction in the marrow in agranulocytosis is rare, and its integration in a scheme for agranulocytosis is arbitrary and not justified, because monocytic leukæmia and acute myeloid leukæmia show the same marrow changes The following case shows that with a monocytosis in the blood a promyelocytic marrow need not necessarily occur. If pathological cases are at all a guide to the origin of cells, monocytes are most probably derived from the medullary and extramedullary reticulum This interesting case, which offers circumstantial evidence on several points, has been published by Tobler and Buser-Pluss (1942) in detail Professor Tobler sent me the marrow smears for an opinion, and I am indebted to him for the clinical data

[illegible]

formed an abscess and needed operation. A fortnight later another smaller abscess developed At nine months he had follicular tonsillitis, three weeks later bronchopneumonia. the fever subsided after four days on sulphathiazole At the age of 10 months once again a sore throat with swelling of the submaxillary glands, four days later bilateral otitis media with tenderness over the left mastoid process. With sulphathiazole he had an uneventful recovery The blood was examined at that time and showed a leucocyte count of 7,250 with only 5% neutrophils and 22% monocytes, and an increase of platelets of 940,000

Repeated blood examinations (during the illness blood counts were made on more than fifty occasions) showed persistent neutropenia and monocytosis. Therapeutic trials with intramuscular injections of blood and intravenous blood transfusion, Campolon, pentnucleotide and nicotinic acid, only resulted in a transient rise in granulocytes, which was most

marked after intravenous transfusion. Even then there were only 12·5% neutrophils of a total leucocyte count of 5,200. Following liver therapy the erythroblasts increased in number (see sternal puncture of May 5th, [illegible]

I [illegible]

the [illegible]

and an increase of neutrophils:—

(1) *During an intercurrent bout of pneumonia, the leucocytes rose* [illegible]

(2) In an attack of scarlet fever the number of neutrophils reached the previously unprecedented figure of 1,591 per cmm. for the first time in six months; eosinophils were 9·5%, monocytes 24%. Within two days [illegible] per cmm while the [illegible] ve give the sternal

TABLE 13

Blood and Marrow Findings in Neutropenia (Case 36)

Blood	Normals	3 3	26 5	29 5	7 7	7 10
Leucocytes per cmm		7,900	6,000	5,200	7,500	
Basophils %		—	0 5	0 5	0 5	0 5
Eosinophils %		0 5	5 0	—	22 5	32 0
Neutrophils %		1 0	4 0	12 5	5 0	5 0
Lymphocytes %		83 5	73 0	53 5	58 0	53 0
Monocytes %		14 0	16 0	31 5	13 0	5 5
Plasma cells %		1 0	—	—	1 0	0 5
Marrow		31 3	5 5	29 5	7 7	7 10
Proerythroblasts per 100 white cells	0 8	0·3	1 3	0 3	0 6	0 6
Early normoblasts per 100 white cells	3 2	0 6	0 3	0 6	1 3	1 6
Normoblasts per 100 white cells	24 4	15 6	50 6	33 6	28 6	38 6
Myeloblasts %	1 2	0 6	1 6	2 3	1 0	1 3
Promyelocytes %	2 2	0 3	3 6	6 6	2 3	3 0
Semimature myelocytes %	5 4	1 0	3 6	13 0	7 0	5 6
Mature myelocytes %	7 2	20 3	11 6	14 3	15 6	7 6
Metamyelocytes %	10 2	22 0	21 3	19 6	15 3	18 0
Stab forms %	24 0	16 3	25 0	16 0	18 6	17 6
Segmented polymorphs %	28 4	10 0	11 0	2 3	12 2	11 0
Eosinophil myelocytes %	1 4	1 6	4 0	1 0	4 3	6 3
Eosinophil metamyelocytes %	1 5	1 6	4 0	1 0	7 0	11 0
Eosinophils %	—	—	—	—	—	9 6
Basophil myelocytes %	0 02	—	—	—	0 6	0 6
Basophils %	0 02	—	—	—	—	0 6
Lymphoblasts %	1 0	—	—	—	—	—
Lymphocytes %	7 6	19 6	9 0	13 3	10 6	6 6
Monoblasts and monocytes %	1 4	0 6	0 3	1 0	0 3	0 6
Megakaryoblasts and megakaryocytes %	0 8	0 6	0 3	0 3	0 6	0 6
Plasma cells %	1 0	1 3	0 3	0 3	0 6	1 3
Lymphoid reticulum cells %	1·0	—	0 6	0 3	0 3	0 6
Phagocytic reticulum cells %	0·2	1 3	0 6	0 3	0 3	0 3
Endothelial cells %	0 8	—	—	—	—	0 3

The peroxidase reaction was positive at every sternal puncture. Toxic changes were noted on March 31st and markedly on May 29th, e g ,

enlargement of nuclei, loss of definition in the chromatin network, vacuolation of nuclei and of the cytoplasm, atypical mitotic figures leading to binuclear cells, reduction in the amount of granulation.

This case was thus one of chronic agranulocytosis. There was a monocytosis in the blood, but the marrow showed no increase of promyelocytes or promonocytes Intercurrent infections increased the monocytosis considerably (up to 35%). Morphologically and also as regards their defence mechanism (bacterial phagocytosis), the monocytes behaved normally. The theory of special "histio-monocytes" in the sense expressed by Rohr, therefore, does not appear to be justified We prefer to follow Tobler's view and assume that monocytes may enter the blood stream from their place of origin, the medullary and extramedullary areas of the

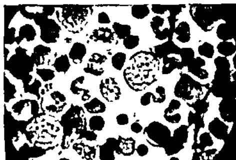


Fig 152 Sternal marrow in chronic neutropenia (agranulocytosis), showing an almost normal marrow, with only a slight shift to the left (× 600)

reticulo-endothelial systems and may be used in the body's defence mechanism They appear to take the place of the neutrophils, when that system is depressed, and in suitable circumstances may increase to meet a demand actually directed at the neutrophil series. The eosinophilia in the last blood picture reflected an eosinophilia in the marrow This case was not so much one of granulocytopenia, but rather one of neutropenia. The basophil series was not disturbed, and the eosinophil series was even hyperplastic, and according to Tobler's investigations took part in phagocytosis We examined the sternal marrow five times between March and October At the beginning there was a myelocytic shift to the left without increase of promyelocytes, but on May 29th the myeloblasts (2 3%) and promyelocytes (6 6%) increased slightly. There was always a sufficient number of cells ready for release into the blood stream (stab forms and segmented polymorphs) Towards the end of the investigations the marrow became relatively normal with 17 6% stab forms and 11·6% segmented polymorphs, and only a slight metamyelocytic shift to the left remained (Fig. 152) Neutropenia was stationary after ten months at a level of between 3% and 6%.

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Lymphoblasts %	1 0	—	—	—	—	—
Lymphocytes %	7 6	19 6	9 0	13 3	10 6	6 0
Monoblasts and monocytes %	1 4	0 6	0 3	1 0	0 3	0 6
Megakaryoblasts and megakaryocytes %	0 8	0 6	0 3	0 3	0 6	0 0
Plasma cells %	1 0	1 3	0·3	0 3	0 6	1 3
Lymphoid reticulum cells %	1 0	—	0 6	0 3	0·3	0 6
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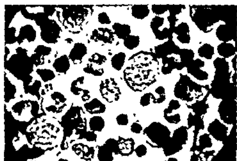


FIG 152 Sternal marrow in chronic neutropenia (agranulocytosis), showing an almost normal marrow, with only a slight shift to the left (× 600)

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Because eosinophilia (up to 38%) was present at the same time and because the mature eosinophil cells entered the blood stream, we must presume that the eosinophil series have their own mechanism for maturation, and for release from the marrow which is completely independent of the neutrophils. We term this phenomenon "dissociated granulocytic reaction." Neutropenia in the presence of a relatively mature marrow suggests a disturbance of the release mechanism. This theory is strengthened, because Tobler and Buser-Pluss could not demonstrate any increase in the leucocytolytic power of the serum in their patient with agranulocytosis. Rapid destruction of the neutrophils, which had reached the blood stream, was thus excluded. It is just possible that the rate of destruction of the leucocytes in the spleen might have been increased. The child is now doing very well up to 1948 and compares favourably with his healthy playmates. The cause of this neutropenia has remained obscure but the repeated infections may have played a part, and possibly also the administration of 20–25 g. sulphathiazole. According to Strong (1941), when the marrow is already damaged by infection relatively small doses of sulphanilamide may lead to agranulocytosis.

This case illustrates many points It shows that normal life is quite possible with very few neutrophils, especially when monocytes and eosinophils take over their function. It also makes a valuable contribution to the problem of the development of monocytes, and to the problem of the mechanism of maturation and release from the marrow of the granulocytes

Rohr's second type is frequently less favourable, as exemplified by the following case :—

Case 37. L. B, a man of 45 years, suddenly fell ill with signs suggesting acute septicæmia. He died within a few days The blood picture showed a typical agranulocytosis with 800-1,000 white cells and absence of granulocytes.

STERNAL MARROW. Early basophilic normoblasts 0 3, normoblasts

This case showed a promyelocytic and immature myelocytic marrow (49%) also involving an increase of myeloblasts and mature myelocytes, but the mature granulocytes were almost completely absent. There was a slight increase of reticulum cells.

Rohr's types 3 and 4 have a very unfavourable prognosis, although occasional remissions may occur Type 3 with acellular marrow and hyperplasia of the reticulum is characteristic of idiopathic or essential agranulocytosis (Kienle, 1943) Like Ferrata

(1935) he distinguishes two types, (1) drug agranulocytosis, and (2) toxic agranulocytosis and states that differentiation is possible only on the basis of sternal marrow biopsy. Drug agranulocytosis, which is equivalent to Rohr's type 2, is accompanied by a shift to the left in the granulocytes, but in toxic agranulocytosis the bone marrow is aplastic. In our experience the marrow pictures may not differ in essential and in symptomatic agranulocytosis, but type 2 with the shift to the left is slightly more frequent in drug agranulocytosis. Erythropoiesis and thrombocytopoiesis are affected more often in the symptomatic than in the idiopathic forms. Type 3 may undergo cure, but this is rare (Nordenson, 1936, Rohr, 1939; Thaddea, 1941; Heilmeyer, 1942) Case 43 (p. 266) illustrates such a relatively benign course.

The fourth type of marrow reaction with an aplastic fatty marrow in which even reticulum cells are very scanty, has by far the least favourable prognosis. The reason for this is the importance of reticulum cells for cellular and humoral immunity (Leitner, 1945). Our experience tallies with that of Jasinski (1944) and others.

In the fifth type the cause suggested is an increased peripheral leucocytolysis. Francke (1940) attributes this to a factor in the serum. He has observed more rapid destruction of leucocytes in the serum of patients with agranulocytosis than in the normal serum Some time ago we recorded similar observations (Table 14),

TABLE 14

Leucocytes and Serum in Agranulocytosis

	Normal leucocytes		Leucocytes from cases of agranulocytosis	
	At room temperature	In the incubator	At room temperature	In the incubator
Normal serum	slight damage	slight damage	slight damage	somewhat more marked damage
Serum from cases of agranulocytosis	rapid destruction	rapid destruction	rapid leucocyto-lysis	even more marked leucocyto-lysis

and Thaddea (1941) has reported similar results. In other cases, Roberts and Kracke (1930), Tobler and Buser-Pluss (1942) and we ourselves have obtained negative results The sudden disappearance of the cells from the peripheral blood can only be explained by the increased rapidity of destruction of the leucocytes. Jasinski presumes that the destruction of leucocytes occurs in the bone marrow, where they are phagocytosed by the reticulum cells. It is probable, however, that increased peripheral destruction of leuco-

cytes accounts for at least some part of this phenomenon. Though we agree with Jasinski that the phagocytosis of leucocytes in the marrow is very rapid, it can only occasionally be demonstrated by sternal puncture which is not what we would expect if it were an important cause. We have frequently looked for this phenomenon, but have never observed it to a degree which might explain such a sudden disappearance of leucocytes from the blood stream. Possibly the leucocytes in cases of agranulocytosis are less resistant than normal. Astaldi's (1941) observation of the rapid death of granuloblastic marrow in marrow tissue cultures in cases of myelopathies is in favour of this view. In primary splenic neutropenia (p. 246) marked phagocytosis of leucocytes in the spleen has been described.

It is quite clear that not only may marrow reactions of different types occur in different cases, but they may successively occur in one and the same case. When some noxious cause continues to act, a type 2 or 3 may undergo transition into type 4. Regeneration during recovery may occur similarly The cells are still able to divide in the promyelocytic marrow judging by the cellularity of the marrow and the relative frequency of mitoses, though these are often atypical. This ability may ultimately be damaged as well, while in other cases the only change is an arrest of maturation. Jasinski points out that division, maturation and growth of cells do not always run parallel We have observed this fact in several varieties of myelopathy. These processes are, however, geared together. Following abnormal cell division, especially after a dissociation of nuclear and cytoplasmic division (Leitner, 1941) or with multipolar karyokinesis, the cells become large without any impairment of maturation. Relevant photomicrographs are given in Chapter IV. The sensitivity of the various cell components may differ from disease to disease, possibly depending on the nature of the individual noxious agent at work. Sometimes disturbance of cell division predominates and at other times a disturbance of maturation. Cell growth does not appear to be of any nosological importance. The sensitivity of the cells at the individual stages of maturation remains constant The destruction of the cells of the various maturational stages proceeds in agranulocytosis on the same lines as occurs in post-mortem changes (p. 22) (Jasinski, 1944), and in marrow infected *in vitro* (Galinowski, 1939 , Leitner, 1941)

The prognosis of agranulocytosis is serious with a mortality of 70%–80%. Therapeutically, stimulating doses of X-rays have been recommended by Friedemann (1928) and Thaddea (1941), transfusions of blood from pyrexial patients by Lamer (1937) and from cases of leukæmia by Schittenhelm (1925), Bock (1937) and Deglmann (1937). Baumann (1938) gave extracts of red bone marrow and Conner *et al.* (1932), Marberg a[illegible] (1938) and Giffin and

Watkins (1938) of yellow bone marrow; Jackson *et al.* (1932), Jackson and Tighe (1939) and Whitby and Britton (1946) gave pentnucleotide. Bock and Wiede (1930), Seiler (1935) and Roth (1938) produced artificial abscesses. None, however, of these methods has fulfilled the hopes placed in them.

Repeated blood transfusions (Rohr, 1939, Leitner, 1941; Heilmeyer, 1942) were probably the best treatment until the introduction of the sulphonamides and penicillin for preventing the onset of infection. Dameshek and Wolfson (1942) and Nixon *et al.* (1943) suggested the combat of infections by sulphonamides. But the dangerous sulphonamides have been superseded by penicillin, of which 150,000–500,000 units, or even more, are given daily. Almost all cases of acute agranulocytosis can be saved, especially when treated early enough by the use of penicillin. Remissions, both of the peripheral blood and of the marrow, proceed rapidly. Tyson, Vogel, Rosenthal (1946), Michaud (1946), Bickel (1946), Urbach and Goldbergh (1946), Mackenzie (1947). However, Spain and Clark (1946) report a case which was apparently due to penicillin sensitization Cantor and Scott (1945) and others found intravenous pyridoxine effective in agranulocytosis due to thiouracil.

Summary. A distinction must be drawn between acute and chronic agranulocytosis. Sternal marrow biopsy has produced some valuable points for its diagnosis and prognosis and also has advanced our knowledge of the course of agranulocytosis as studied at the site of origin. It is universally recognized that prognosis in cases of agranulocytosis with only slight changes in the marrow picture, such as slight shift to the left, is favourable. Considerable disturbance of maturation (type 2) with promyelocytic marrow is more serious and aplastic marrow reactions (types 3 and 4) are frankly unfavourable Extreme caution in assessing prognosis is indicated, especially in view of the lack of homogeneity of the marrow. In types 3 and 4 it must be remembered that owing to poor cellularity disappearance of the granuloblasts may simulate reticulum cell proliferation, even if the more resistant reticulum cells are normal in number or even diminished. Recovery is possible even in types 3 and 4, though much rarer than in types 1 and 2. Provided the sources of error are kept in mind, sternal puncture is of very great value in forming an opinion on any given case of agranulocytosis It also allows a better insight into the pathological processes involved

ALEUKIA HÆMORRHAGICA (FRANK) AND PANMYELOPHTHISIC ANÆMIA (APLASTIC ANÆMIA)

Isolated damage to granulocytopoiesis is not at all common (Fatzer, 1936, Staehelin, 1938). It is usual for other systems to be affected also By aleukia hæmorrhagica we mean a disease involving

two systems with damage to granulocytopoiesis and to thrombocytopoiesis; anæmia may be present at the same time. When the anæmia is due to hæmorrhage caused by lack of platelets we are entitled to speak of a disease affecting two systems. By panmyelophthisic anæmia we mean a marrow deficiency involving all three systems producing agranulocytosis, thrombocytopenia and anæmia.

Much confusion persists in the terminology of the names of these diseases. For panmyelophthisic anæmia, and more especially for its chronic form, the term "aplastic anæmia" coined by Ehrlich (1888) is used widely. Stodtmeister and Büchmann (1941) speak of "aplastic" or "essential marrow insufficiency" and, when the disease takes an acute course, of "aplastic crisis" Hoff (1942) groups all these pathological processes (agranulocytosis, aleukia, aplastic anæmia) together under the name "*myeloid insufficiency.*" Marrow deficiency is not always present when cells are decreased in number in the blood stream, and therefore Stodtmeister and Buchmann (1941), Glanzmann (1942), Nordenson (1943), Palmén (1943) and others prefer the term "panhæmocytophthisis." Now that we can assess by sternal puncture what is actually going on in the marrow, we prefer the terms "panmyelopathy" and "panmyelophthisis" qualified as required by the adjectives "acute" and "chronic." The cases with general deficiency of cells in the blood, but *without* marrow deficiency, are designated panmyelopathy, and those *with* marrow deficiency are panmyelophthisis. We feel these terms are more appropriate and reflect the state of the tissue which is the seat of the trouble, more accurately.

Ætiologically various noxious factors need to be considered: infections, drugs, such as gold, salvarsan, quinine, industrial intoxications (especially benzol), X-rays, radium and a host of others Frequently the actual cause cannot be found, and such cases may be called "essential or idiopathic" Just as in the case of agranulocytosis there may be acute and chronic forms. At first only one or two systems may be involved, but later all the other systems may be affected Though certain noxious agents (e g, amidopyrine) consistently affect one system only, as a matter of principle there is no fundamental difference between agranulocytosis and panmyelopathy. For diagnosis the raised serum iron is important in early cases (Heilmeyer and Plötner, 1937; Stodtmeister and Buchmann, 1941) Marrow findings may be divided into five types —

(1) Slight changes with moderate shift to the left of granulocytopoiesis, and moderate hypoplasia of erythropoiesis and thrombocytopoiesis.

(2) Advanced maturation arrest with shift to the left of the granuloblasts up to a promyelocytic type of marrow, frequently with an increase of myeloblasts; shift to the left of the marrow giant

cell system, with scanty or even absent platelet-forming megakaryocytes, and hypoplasia of erythropoiesis.

(3) Aplasia of the cell-forming marrow with marked decrease of granuloblasts, erythroblasts and megakaryocytes (which may disappear almost completely), while reticulum cells are increased.

(4) Total marrow aplasia, in which not only the cell-forming marrow of the three systems, but also the reticulum cells disappear.

(5) Damage in the peripheral blood with destruction of leucocytes, erythrocytes and platelets in the spleen.

This destruction appears to occur to some extent in the other four types also, but it becomes a dominant factor in primary splenic neutropenia. As hyperfunction of the spleen seems to exert an inhibitory influence on the bone marrow these forms of myelopathy cannot be regarded as exclusively peripheral. The individual systems do not always react similarly and we have described several "*dissociated reactions*": *one system may react according* to types 2 or 3, and another according to type 1. In practice the classification outlined above has proved to be more satisfactory than Heilmeyer's scheme, which relies on states rather than types of reaction. As states may occur in innumerable variants, his scheme is rather confusing and arbitrary, and it is often difficult to classify cases under his headings.

In type 1, in opposition to Kiyono and Amano (1937) and Tanaka, Miyake, Takaki, Ishii and Hirayama (1937), we believe that there is a more or less definite disturbance of maturation, as well as a disturbance of the release mechanism.

sensation of pressure in the left epigastrium, and with pyrexia. Hæmatogenous dissemination throughout both lungs and a large spleen reaching to the iliac crest were found. A cervical gland, taken for biopsy, showed caseating tuberculosis. Anæmia, leucopenia, thrombocytopenia were present; splenectomy was performed for these reasons. She then spent eighteen months in a sanatorium, which led to recovery sufficient to enable her to work again. Later another bout of hæmatogenous dissemination occurred with swelling of a knee joint, tuberculosis of the skin, of a rib, and of many lymph glands.

Blood Count Before Splenectomy. R.B.C. 3.5 millions, Hb 76% = 12.2 g%, W.B.C. 3,400; basophils 1%, eosinophils 3.5%, segmented polymorphs 77%, lymphocytes 12.5%, monocytes 6%, platelets 76,000; reticulocytes 1.6%.

Sternal Marrow Before Splenectomy. Proerythroblasts 0.3, early basophilic normoblasts 6.3, normoblasts 20.6 per 100 white cells, myelo-

megakaryocytes 1%, plasma cells 1·3%, primitive reticulum cells 1%.

... R.B.C. 3·8 millions, Hb. ...phils 0·5%, eosinophils 3·5%, ... forms 1·5%, segmented polymorphs 78%, lymphocytes 10·5%, monocytes 6%. Later: R.B.C. 4·4 millions, Hb. 87% = 13·9 g.%; platelets 295,000. After splenectomy the number of semimature and mature myelocytes in the marrow fell to 8·3% and 10% respectively, and megakaryoblasts and promegakaryocytes fell to 0·3% and 0·6% respectively.

In this case of caseating tuberculosis of lymph glands, multiple hæmatogenous dissemination into various organs and tuberculous splenomegaly, owing to hyperfunction of the spleen moderate anæmia, leucopenia and thrombocytopenia occurred and these improved after splenectomy.

Other authors have recorded similar cases of panmyelopathy from inhibition of the marrow induced by a splenic toxin. Ferrata and Fieschi (1941), Selander (1942) and Whitby and Britton (1946) and others therefore suggested splenectomy for the treatment of aplastic anæmia, just as Hegler (1938) and Reissmann (1938) suggested it for agranulocytosis. Weyeneth (1942) described a remarkable case of atrophic thyroiditis with splenomegaly which caused inhibition of the bone marrow. Doan and Wright (1946) recorded several cases of "primary congenital, and secondary acquired splenic panhæmatopenia" Splenectomy leads to a permanent cure. By the use of supravital preparations applied to splenic pulp material immediately after removal they observed that the splenic macrophages showed an increased amount of phagocytosis of the blood cells.

Case 39. A woman of 24, as a school child had discharging tuberculous cervical lymph glands; pleurisy in 1935; since January, 1938, lassitude, loss of appetite, abdominal pain When examined, she had groups of enlarged glands in both sides of the neck and extensive infiltration in the right mid-zone, the abdomen was enlarged and tense with extensive ... and ascites

... 2·9 g.%, W B C 6,600; ... orphs 76%, lymphocytes ... 1/61 mm. Later R.B C 2·82 millions, Hb. 60% = 9·? g %, W.B C ?,?20, platelets 120,000

...

lymphocytes 3·6%, monocytes ?%, megakaryocytes ... 5·6%, reticulum cells 1·6%, endothelial cells 0·6%

This is a case of generalized, caseo-calcareous tuberculosis of the lymphatic system with anæmia, leucopenia and thrombocytopenia. Sternal puncture showed hypoplasia of erythropoiesis and a myelocytic shift to the left, without damage to thrombocytopoiesis

The shift to the left of the granuloblasts in the marrow was quite considerable and in conjunction with the peripheral leucopenia had to be attributed to marrow inhibition on a splenopathic basis.

Case 40. M. H., a schoolgirl of 15, in the spring of 1940 fell ill with primary tuberculous infection with erythema nodosum. In November, 1940, tubercle bacilli were found in the sputum, and she was admitted to the sanatorium. When examined she had extensive bilateral open pulmonary tuberculosis, generalized swelling of the lymph glands and enlargement of the liver and spleen.

FIRST BLOOD COUNT (November 26th, 1940) R.B.C. 3 [illegible] Hb. 65% = 10·5 g %, W.B.C. 15,250; basophils [illegible] stab forms 4%, segmented polym[illegible] 10%, plasma cells 2%;

SECOND BLOOD COU[illegible] Hb 38% = 6·1 g.%, W[illegible] R.B.C. 2 3 millions, 17 5%, segmented polymorphs 66 5%, lymphocytes 7·5%, monocytes 7%; platelets 38,000, bleeding time prolonged. Clinically, hæmorrhagic diathesis with hæmorrhages into skin and mucous membranes.

1ST STERNAL PUNCTURE (January 29th, 1940) Early basophilic normoblasts 0 3, normoblasts 6·3 per 100 white cells; shift to the left of granuloblasts with 14 6% promyelocytes, 15% semimature and 14·3% mature myelocytes Plasma cells increased.

2ND STERNAL PUNCTURE (immediately after death in January, 1941). Early basophilic normoblasts 0 3, normoblasts 3 3 per 100 white cells; even more pronounced myeloid shift to the left with 17% promyelocytes, 1[illegible] semimature and 20% mature myelocytes Hypoplasia of marrow giant cells (megakaryoblasts 0 3%, megakaryocytes 0 6%, none being of the platelet forming variety) Autopsy confirmed the clinical diagnosis of generalized caseating tuberculosis of the lymph glands, tuber[illegible] of spleen and liver.

This also was a case of generalized caseating tuberculosis of the lymphatico-hæmopoietic system, occurring as a sequel of a [illegible] primary infection. The blood showed a progressive anæmia [illegible] thrombocytopenia of 38,000, which led to a hæmorrhagic diathesis. Leucopenia, on the other hand, did not occur; initially there was even leucocytosis. The sternal marrow showed hypoplasia of erythropoiesis, increasing up to the time of death, and a shift to the left of the granulocytic series, also increasing up to the time of death. Promyelocytes and myelocytes amounted to 55 6% of the marrow cells

These 3 cases have several features in common In the [illegible] there was hypoplasia of red cells and megakaryocytes, [illegible] granulocytopenia, but instead a lymphopenia All three patients suffered from a severe disease—tuberculosis. Only the [illegible] enjoyed a remission lasting four years, following [illegible] In the third case there was no leucopenia, but thrombocytopenia was so severe that it led to hæmorrhagic diathesis In [illegible] marrow none of the patients showed aplasia, but [illegible] plasia of erythropoiesis. The shift to the left of the granuloblasts was particularly marked in the third case A more [illegible]

maturation arrest of the granuloblasts was a common feature. In the cases with leucopenia the inhibition of the marrow was probably caused by the spleen and in the cases with leucocytosis a simple shift to the left of infectious origin cannot be excluded. The large tuberculous spleen found at post-mortem would not, however, favour this theory in our case.

For the second type of marrow reaction the following case of acute panmyelopathy is an instructive example. It showed a considerable degree of maturation arrest at the level of the myeloblasts and promyelocytes.

epistaxis and a week later he was admitted to hospital. Epistaxis recurred. The day after admission, while trying to get out of bed, he collapsed and lost consciousness. When examined, he continued to bleed from the nose in spite of various attempts to stop it, skin and mucous membranes were

oculi pale, optic papillæ not unusually pale, veins engorged, several flame-shaped and patchy hæmorrhages Œdema and punctate hæmorrhages in the region of the macula In the temporal half, hæmorrhages in the deeper layers. Blood pressure 138/60; apical systolic murmur. Spleen and liver not enlarged on palpation Lungs and kidneys, normal, temperature 104° F.

BLOOD. R B.C. 2 0 millions, Hb 41% = 6 6 g.%, C I. 1·2, W.B.C. 660; eosinophils 1%, immatur
19%, lymphocytes 61%, mon
polychromasia, 1 normoblast

rea 32 mg %, serum cholesterol
%, serum calcium 10 2 mg.%;
plasma proteins 7 63 g.% Therapy consisted of a blood transfusion of 350 ml, nucleotrat (pentnucleotide), calcium, redoxon, coagulen and manetol (an extract of spinal cord, used for hæmostasis) The day after
W.B C. 1,170,
velocytes 23%,
20%, monocytes
ablished by the
positive oxidase reaction. The anæmia was ... improved Hb. 50% = 8 g.%, R B C 2·1 millions, C I 1 19, polychromasia, anisocytosis, poikilocytosis, 8 normoblasts per 100 white cells.

STERNAL MARROW (Figs. 153–160) (the marrow clotted very rapidly). Early basophilic normoblasts 2·5, normoblasts 15 per 100 white cells; myeloblasts 17%, promyelocytes 51%, semimature myelocytes 2 5%,
ented
myelo-
25%,
75%
there
was marked tendency to lobation, and they had many ... nuclei

Promyelocytes showed large nucleoli (Figs 156–159), their cytoplasm

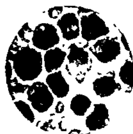


Fig. 153



Fig 154



Fig 155



Fig. 156

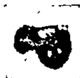


Fig 157



Fig. 158



Fig 159



Fig 160

Fig 153 Myeloblastic promyelocytic marrow in panmyelopathy (× 500)

Fig 154 Myeloblasts, some with tendency to lobation in the sternal marrow in panmyelopathy (× 500)

Fig 155. Promyelocytes in sternal marrow in panmyelopathy (× 1,000)

Fig 156 Tendency to lobation of promyelocytes in sternal marrow in panmyelopathy (× 1,000)

Figs 157–159 Myeloblasts (paramyeloblasts) with much lobation in sternal marrow in panmyelopathy (× 1,000)

Fig 160 Megakaryocyte in histological section of femoral marrow in panmyelopathy (No megakaryocytes in sternal marrow) (× 500)

The course was steadily downhill The hæmorrhages ceased temporarily following transfusion, but recurred later and resisted all forms of therapy. On the third day the patient complained of headache and

developed a left-sided hemiplegia, and he died of cerebral hæmorrhage the same evening. Autopsy (Pathological Institute of the University of Berne) confirmed panmyelopathy with multiple hæmorrhages in the skin and mucous membranes, *pericardium, pleura, stomach, bladder,* retina and brain Also pneumonia and splenomegaly; both in the sternum and in the femur the marrow was red throughout

The blood picture was thus one of panhæmocytophthisis with extreme leucopenia (660!), *granulocytopenia* (19% segmented polymorphs, 61% lymphocytes), thrombocytopenia (10,000) and anæmia, which, of course, was *partly due to loss of blood. Sternal* puncture showed no evidence of myelophthisis but a maturation arrest of leucocytes and hypoplasia of the *red series and of the* megakaryocytes. The maturation arrest was at the myeloblast (17%) stage and there were also 51% *promyelocytes, most of them paraforms, which were oxidase positive. The reticulum* cells were increased and many were binuclear. There was therefore dissociation of nuclear and cytoplasmic division as well as of nuclear and cytoplasmic maturation. The release of immature cells into the blood stream was remarkable: 9·5% paramyeloblasts, 7·5% promyelocytes, 23% parapromyelocytes, 26% myelocytes, resulting in a blood picture almost indistinguishable from leukæmia We interpreted this as a terminal breakdown of the marrow-blood barrier, especially as two days before there were only 7% immature cells in the blood. It is possible that paraforms may be able to pass this barrier more easily than the forms with round nuclei since their shape is more like that of the segmented polymorphs. This might explain the developments of paraforms as a purposeful process after all. However, these immature forms are not fully capable of assisting the body's defence mechanism, and with Stodtmeister and Büchmann (1941) one could apply the term "frustrated attempt at compensation." *When immature forms first appeared in the peripheral* blood all the cells showed marked lobation, but terminally even *myelocytes with round nuclei and also 8% normoblasts were found* Since extramedullary hæmatopoiesis could not be demonstrated at post-mortem material *a terminal breakdown of the marrow-blood* barrier was postulated

Rohr (1936), Rhoads and Miller (1938), Heilmeyer (1942), Thaddea (1943), Kienle (1943) and many others have recorded similar marrow findings. According to Fieschi (1940), a hypoplastic rather than an aplastic marrow is usually found His first case showed only 2% erythroblasts, 56% lymphoid cells and 23% plasma cells, and the second case a progressive marrow atrophy with 10 2% normoblasts, which could therefore be called a "reticular marrow" In Case 41 (p 260) the marrow was slightly hypoplastic. Gerlach (1932), Rohr (1936), Schulten (1937), Israëls and Wilkinson (1938), Schultz (1938), De Weerdt (1939), Henning and *Keilhack* (1939), Hynes (1939), Stodtmeister (1940), Kimura and Kumagai (1941),

Leitner (1941), Heilmeyer (1942), Davis and Davidson (1944) and others also reported cases with hyperplastic marrow.

Rhoads and Miller (1938) in 69 cases found only 28 with marrow hypoplasia, and some of these had a definite increase of megakaryocytes. The differentiation of myelopathies which are accompanied by an immature hyperplastic marrow and an immature blood picture, from acute leukæmia, is extremely difficult, if not impossible (Muralter, 1933; Schulten, 1937; Grunke, 1938; Henning, 1936; Stodtmeister and Buchmann, 1941; Hoff, 1942; and others). According to Bakalos and Thaddea (1943) such differentiation is often artificial or arbitrary.

The third form of marrow reaction is characterized by the fact that cells are very scanty. Sternal puncture is often difficult, only little material is obtained, and very few grey marrow fragments are found (Weil, Isch-Wall and Perlès, 1938; Leitner, 1941; and Nordenson, 1943). Naegeli, Rohr and Henning report marrows which were very fatty, with lymphoid elements, plasma cells, fat cells and reticulum cells. The parenchyma is atrophic also (Osato, Hashimoto and Takigawa, 1931; Rohr, 1936; Behr, 1937; Francke, 1940; Leitner, 1941; Poli, 1941; Bakalos and Thaddea, 1943). Nordenson recorded cases of atypical aplastic anæmia with myeloblastic degeneration, in which the blood picture showed anæmia with erythroblastosis (0.5%–34.5%), thrombocytopenia, and leucopenia with 6%–40% myeloblasts. The marrow, however, was aplastic and there were no extramedullary hæmopoietic foci. Case 41 (p. 260) is very similar to this case as far as the blood picture is concerned, but the marrow was not aplastic. The question arises as to the origin of the immature cells in the blood in Nordenson's cases since the marrow was so aplastic and there was no extramedullary hæmopoiesis. We suggest that the marrow was not aplastic throughout and that this well-known lack of uniformity of the marrow may have led to a misinterpretation. January and Fowler (1940), found in most of their cases aplasia of the marrow at post-mortem, but also found islets with well preserved marrow tissue. In both Nordenson's and our cases the hiatus leukæmicus was missing, which is an important point in the differential diagnosis from acute myeloid leukæmia. The third type of marrow reaction with aplasia is panmyelophthisis proper and the prognosis is unfavourable (Domarus, 1937; Lupu, 1940), but cures have been reported. Nevertheless, extreme caution is indicated when estimating prognosis on the basis of marrow pictures. The following case is typical of panmyelophthisis with marrow aplasia:—

Case 42. D. H., an apprentice, aged 16, suddenly became increasingly pale. Because he looked ill, the mother took his temperature, which was 103° F. Next day his nose bled and he was admitted to the clinic with the diagnosis of purpura hæmorrhagica. When examined he was pale and cyanosed. There was blood coming from the nose and

developed a left-sided hemiplegia, and he died of cerebral hæmorrhage the same evening. Autopsy (Pathological Institute of the University of Berne) confirmed panmyelopathy with multiple hæmorrhages in the skin and mucous membranes, pericardium, pleura, stomach, bladder, retina and brain. *Also pneumonia and splenomegaly; both in the* sternum and in the femur the marrow was red throughout.

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pharynx and there were bluish areas of hæmorrhages on arms, legs, back and abdomen. Temperature 104° F.; pulse 130; heart, normal; spleen enlarged, as estimated by percussion. Urine: albumen 0·1 g.%, deposit of R.B C. and leucocytes

BLOOD. R.B.C. 1·6 *millions*, *Hb.* 37·8% = 6·1 g.%; *C* I. 1·1; W.B C. 480; metamyelocytes 1%, stab forms 3%, lymphocytes 96%. No segmented polymorphs. No platelets seen. *Bleeding time 15 mins.*; clotting time 10·5 mins.; reticulocytes 0·1%; anisocytosis, polychromasia; clot retraction delayed. Sedimentation rate (Westergren) 67–136–160 mm (1, 2 and 3 hr.).

STERNAL MARROW. Poorly cellular marrow, with a total of nucleated

2·25%, plasma *cells* 7%. No megakaryocytes seen.

In spite of prompt blood transfusion, the leucocytes fell to 417 the ... R.B.C 2·1 *millions*, Hb. 40% =

... ie University of Berne). Aplasia

diathesis, no extramedullary foci of hæmopoiesis.

This was the blood picture of panhæmocytophthisis with *only 480 leucocytes, 96% being lymphocytes, and without mature* granulocytes, 1·6 millions erythrocytes, and a complete absence of *platelets The hæmorrhagic diathesis, just as in the previous case,* dominated the clinical picture There was thrombocytopenic *purpura with prolonged bleeding time Sternal puncture and his*tological examination of bone marrow obtained at autopsy showed a *picture of panmyelophthisis with aplasia of granulocytopoiesis* and thrombocytopoiesis. In the sternal marrow there was *still a relatively high* number *of erythroblasts, but owing to the extreme* scarcity of cells they were diminished in absolute number. In the aplastic marrow, *reticulum cell hyperplasia was observed (Figs* 161 and 162)

The eosinophilia in the marrow of 15·5% without eosinophilia in the blood was noteworthy. According to Rohr this indicates a reaction of *hypersensitivity, and warrants a favourable prognosis*, but this was not borne out in our case. Though in various papers *we have expressed the view that eosinophilia indicates hypersensitivity* our case rather suggests an increased resistance of the eosinophils against *whatever noxious agent may have been at work as* compared with the neutrophil *polymorphs* The relatively large number of *erythroblasts may be explained similarly*, but some disturbance of maturation (faulty disposal of the nucleus) must

have been present as well, because the number of reticulocytes was very low, being only 0·1%.

In this case all types of reaction were present. (1) Aplasia

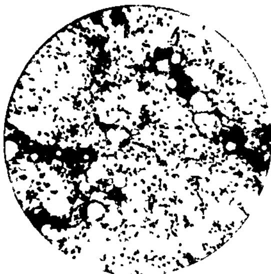


FIG 161 Sternal marrow in panmyelophthisis Poorly cellular marrow. (1 × 120)



FIG. 162 Sternal marrow in panmyelophthisis Reticulum cell proliferation in a poorly cellular marrow (× 1,000)

of thrombocytopoiesis and neutrophil granulocytopoiesis (2) Disturbance of maturation of erythropoiesis (faulty disposal of nuclei). (3) Disturbance of the release mechanism for the

eosinophils. The extramedullary lymphocytes also must have been more resistant than normal; their number was relatively high, but the absolute figures were reduced also. This observation teaches us that the various blood cell systems *may have different powers of* resistance, but their reactions follow the same fundamental laws. How far the individual systems take part in these abnormal or *aplastic reactions depends on the general condition* and on the type of noxious agent at work. The three types of granulocytes again vary in their sensitivity; we have pointed this out already in the section on leukæmia and on agranulocytosis ("dissociated granulocytic reaction"). We have observed plasma cell proliferation as well as reticulum cell proliferation, and Wienbeck (1938), Rohr (1940), Heilmeyer (1942) and others have confirmed this. Chronic myelopathies may also lead to a myelophthisic reaction.

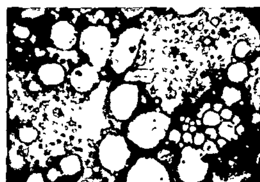


Fig. 163 Sternal marrow in chronic panmyelophthisis Marrow shows only scanty cells. (× 150)

The fourth type is characterized by aplasia of all types of cells and decrease of reticulum cells In the following case the percentage figure of reticulum cells suggests an increase, whereas in actual fact in the extremely acellular *marrow they were decreased* in absolute number (Fig. 163) :—

Case 43. H M., a woman of 36 years, suffered from pulmonary tuberculosis with cavitation of the left upper and mid-zones with foci of dissemination in other parts of the lungs. Apart from this she also had hypochromic anæmia, leucopenia and thrombocytopenia Only transient [illegible] ment with iron, liver, arsenic and by [illegible] osis was *treated by* extra-pleural [illegible] anæmia for a short while only. [illegible] anæmia, R.B C 3 9 millions, Hb. [illegible] il, 1943, R.B.C. had dropped to 2 7 [illegible], W B C. 3,000, eosinophils 1·5%, [illegible] norphs 30 5%, lymphocytes 25 5%, monocytes 12·0 %, [illegible] By May the leucocytes decreased *to 1,600, with basophils 0 5%, eosinophils* 1%, stab forms 23%, lymphocytes 26·5%, monocytes 11%, reticulocytes 4·7%, anisocytosis, polychromasia, platelets 61,000

1st Sternal Puncture Poorly cellular marrow with relatively numerous reticulum cells (Figs 163, 164) Procrythroblasts 0·3, early basophilic normoblasts 1, normoblasts 15 3 per 100 white cells, myeloblasts 0 6%, promyelocytes 1·6%, semimature myelocytes 2 3%, mature [illegible] 11·3% *metamyelocytes* 9 3%, stab forms 9%, segmented [illegible]

cells 32 3%, phagocytic reticulum cells 0 6%. The normoblasts were mainly orthochromatic, with pyknotic nuclei and often with punctate ... the erythroid and myeloid series. Early

The anæmia proved refractory to all therapy (iron, arsenic, liver, transfusions) and even with suitable treatment of the lung condition (pneumolysis) improved only temporarily. It is improbable that the blood dyscrasia was due to tuberculosis. Marrow punctures showed an extremely acellular marrow with hypoplasia of erythropoiesis, leucopoiesis and thrombocytopoiesis, and with the so-called "basophil ball cells" (tissue mast cells), which Undritz (1946) thinks are of diagnostic importance in panmyelopathy. In the marrow smears a high percentage of reticulum cells could be observed, but taken absolutely there must have







Fig. 164 Fig. 165. Fig. 166

Fig. 164 ... in pan-

Figs. 165 ... chronic

panmyelopathy

been a decrease even of these cells, although they suffered the least damage. They had nuclei, which were less juicy and less ... Case 42, but they were dark. Nucleoli

nucleus and coarse, black-purple ... nucleus. The cell outlines were often indefinite (Figs. 165, 166). The number of mitotic figures was decreased markedly in all three cell series, prophases were completely absent. Gasser (1944) published a case of chronic panmyelopathy similar to this case. In his case also the sternal marrow showed hypoplasia of all three systems and transfusions were unsuccessful. The underlying cause was obscure, leukæmia could be excluded because there were no pathological cells in either blood or bone marrow.

The fifth type with peripheral cell destruction may play a sup-

plementary part in every other type, especially as far as platelets and leucocytes are concerned. Owing to the many different ways in which the individual systems may respond, subdivisions of the types of reactions already discussed can be made. Such a scheme is Heilmeyer's who classifies as follows :—

(1) Marrow, showing total aplasia with scanty lymphoid reticulum cells.

(2) Marrow, showing few normoblasts, but with relatively well preserved granulocytopoiesis.

(3) Hypoplastic marrow with decrease of marrow cells and increase of lymphocytes.

(4) Cellular marrow with increase in the precursors of white and red cells.

(5) Decrease of all marrow cells and simultaneous increase of reticulum cells.

The six cases of myelopathy described show that still more types of reaction may be differentiated. Very often, however, they are merely different phases of progress of one or other of the main types. Rhoads and Miller (1938) and also Steinbrinck (1938) (whose case was examined at autopsy and histologically by Wienbeck), reported increased megakaryocytes. This must be a very rare reaction, which so far we have not met Blood and marrow reactions are often very variable, because panmyelopathy may affect the individual systems to a varying degree.

Moeschlin and Rohr (1943) recorded a most interesting case of aplastic anæmia, in which erythroblasts were apparently absent from the marrow for several years. The patient was a girl of twenty years who had fourteen marrow punctures (sternum, pelvis, trochanter and shaft of femur) in two and a half years. They all showed erythroblastic aplasia, with a maximum of 1·2% of sometimes doubtful erythroblasts Reticulocytes were also missing, but granulocytopoiesis was relatively well preserved, except that the mature leucocytes showed clumping of the chromatin The patient was kept alive by altogether 120 blood transfusions, but eventually died of shock and pulmonary œdema during a transfusion. Hurst and Kark (1937) managed to maintain the life of a patient with similar findings with 290 transfusions over eleven years In their case, however, the erythroblasts did not fall to such low levels, but a severe transient agranulocytosis occurred with only 850 white cells, and no granulocytes. Otherwise the two cases were identical in their main features, both developed marrow eosinophilia, and later advanced hæmosiderosis, and both ended by transfusion shock. Esser (1940) reported the case of a child, who was kept alive by repeated transfusions.

It is possible to maintain pa[illegible] 'th normoblastic aplasia by tran[illegible] [illegible]hrocytes, bu[illegible] [illegible]s perish rapidly when transfus[illegible] of agranulc[illegible] [illegible]is indicates the

primary importance of the marrow and the relatively small part hyperfunction of the spleen plays in these cases. Hæmolysis may become quite considerable in time, which is quite feasible, when blood donors have to be changed frequently and hæmolysins are bound to form eventually. Moeschlin and Rohr believe that the ultimate cause is a chronic marrow inflammation on a rheumatic basis and some disturbance of a central regulating mechanism, possibly in the midbrain.

Summary. Sternal puncture is of great value in the diagnosis and the assessment of prognosis of the myelopathies It makes the study of these extremely pleomorphic pathological pictures easier. As causes of the myelopathies, infections, toxins (benzol, salvarsan), allergy and central nervous noxious factors or any of these combined together may have to be considered Marrow findings vary, but for practical purposes the differentiation of four types of reaction is sufficient.

(1) Slight disturbance of maturation

(2) Severe maturation arrest with primitive marrow.

(3) Aplastic marrow with reticulum cell hyperplasia.

(4) Aplastic marrow without reticulum cell reaction

Because the individual cell systems may react so very variably (even within the three series of granulocytes), dissociated marrow reactions may occur. One or other system may remain intact or even undergo hyperplasia, while another system may show hypoplasia or aplasia. The differentiation of some of the cases from acute myeloid leukæmia is often difficult According to Fieschi the presence of hæmohistioblasts and hæmocytoblasts indicates leukæmia, but occasionally myeloblasts have been seen in panmyelopathies. Like other authors, we have observed plasma cell proliferation in myelopathy, which is never present in acute myeloid leukæmia In the aplastic forms of myelopathy, sternal puncture makes differential diagnosis much easier. In myelopathy with a hyperplastic marrow, diagnosis may be difficult, but in our experience has so far always been possible

LEUCOCYTOSIS AND LEUCOPENIA

We still do not know how and why leucocytosis, or for that matter, leucopenia, develop Sabin *et al* (1925) as well as Schilling (1933) believe that the release of leucocytes into the blood stream occurs in intermittent showers According to Doan and Zerfas (1927) maturation of leucocytes is enhanced by an unknown maturation factor chemotactically Menkin and Kadish (1943) have shown that the stimulating factor for leucocytosis found in inflammatory exudates is closely linked to the pseudoglobulin fraction. Leucocytosis is often brought on by peripheral leucocytolysis (Wallbach, 1932, Faludi, 1938). Destruction of

small numbers of leucocytes produces leucocytosis without shift to the left. Severe leucocytolysis causes leucocytosis with a shift to the left if the marrow can muster sufficient reserves, but when the reserve fails, leucopenia develops. It therefore depends on the degree of lysis and on the reserves of the marrow, whether or not a shift to the left will become manifest. Atypical forms of regeneration may develop owing to the action of bacterial toxins, depending in turn on the degree of virulence (Faludi). This suggests a peripheral causation of leucocytosis or leucopenia, just as Gloor (1929) thought was the case with toxic granulation. Cytoplasmic basophilia (Mommsen, 1929; Leitner and Eichhorn, 1932), closely linked with pathological granulation, however, suggests that the latter develops in the bone marrow; (Leitner and Eichhorn, 1932, Stodtmeister, 1938) by dissociation of nuclear and cytoplasmic maturation.

A number of observations suggest that leucocytosis is regulated by the central nervous system. Hoff (1938) believes that leucocytosis accompanied by sudden pyrexia is initiated by the mid-brain. He substantiated this theory by the occurrence of leucocytosis following the introduction of air in ventriculography, and Rosenow (1931) found that leucocytosis did not occur when the cervical spinal cord was severed. When kaolin was injected into the ventricular system of rabbits, Rosenow (1941) observed a definite leucocytosis. The centres regulating the symptoms of leucocytosis, such as pyrexia, the katabolism of proteins and the increase of the basal metabolic rate, are also situated in the midbrain (Isenschmid and Krehl, 1912; Grafe and Grunthal, 1929). The extensive experimental researches of Beer (1938, 1942) favoured the view that the midbrain regulated leucocytosis and that the impulses are conveyed by nervous channels. Using animals living in parabiosis, he ligated all direct communications of blood and other vital juices between them. He then filled the ventricles of one animal with air, and noticed leucocytosis in the other

Hayashida (1935, 1936) produced leucocytosis and erythrocytosis by puncturing the region of the tuber cinereum, and Aburaya (1937) did so by stimulating that area. Schulhof and Matthies (1927) caused erythrocytosis by puncturing the region of the hypothalmic centres. Leucocytosis with a shift to the left was observed by Urra, Baena and Parejo (1934) to follow trauma to various areas of the midbrain Borchardt (1928) suggested that a centre regulating the level of leucocytes was situated in the tuber cinereum in the vicinity of the centres controlling body heat and sweating. Riccitelli (1935) observed that leucocytosis followed injury to the wall of the third ventricle and to the floor of the fourth, but not when other parts of the brain were traumatized. Shinosaki *et al* (1936) applied electrical stimuli to the tuber cinereum and to its nucleus paraventricularis. This was followed

by leucocytosis in which the total number of white cells increased three to five times, with a marked shift to the left. Sakurai (1933) stimulated the tuber cinereum in the region of the nucleus ventricularis and also obtained a leucocytosis with a shift to the left. In some cases irritation of the corpus striatum and of the globus pallidus produced a moderate leucocytosis, but stimuli applied to other areas, such as the cerebral cortex, thalamus, aqueduct of Sylvius, red nucleus, mamillary body or the corpus Luysii, did not give definite results. Wespi's (1944) experiments, in which he applied electrical stimuli subcortically, suggest, however, that the thalamic nuclei also take part in the regulating mechanism

Clinical observations which might throw light on this subject have not as yet been recorded and the existence of a centre regulating leucocytosis in the tuber cinereum is not proven Disease processes selecting this region are extremely rare. Zambelli and Gomirato (1941) examined patients with disseminated sclerosis by sternal puncture and found only a slight disturbance of maturation of the myeloid elements, but in our experience we have not seen any particular marrow changes. Hoff (1938) observed leucocytosis up to 36,000 and a shift to the left in cases of hæmorrhage into the third ventricle, and Högner (1927), Riccitelli (1935), Romcke and Skouge (1931) recorded similar changes Leucocytosis often follows injury to the skull and hæmorrhage into other parts of the brain Hoff, Riccitelli and Wright and Livingstone (1923) believe this may be due to referred impulses According to the two latter authors, leucocytosis following head injury may indicate subdural hæmorrhage, or fracture of the base of the skull, but its absence excludes subdural hæmorrhage. Moser (1930), Riccitelli, Hoff, Gentzen (1934), da Rin and Costa (1934) recorded changes in the white cells in tumours of the midbrain Georgi (1926), Hoff, Tinel and Santenoise (1921) noted that the preparoxysmal leucopenia gave way to leucocytosis in epileptic attacks. Müller and his school (1933) believe that the manifestations of epilepsy are caused by irritation of the midbrain. Hoff, therefore, suggested that the changes in the blood are due to stimulation of the centre regulating the leucocyte levels Wuth (1926) was able to exclude the irritation of the motor area as a causal factor because these changes occurred also with transient disturbance of consciousness and other epileptic-like attacks (Jödicke, 1913, Muller, 1923) We have performed sternal punctures on twelve epileptic subjects, and have observed a shift to the left of the granular series in three patients examined after an acute attack Moser (1930), Reinhart (1922), Stern (1928), da Rin and Costa (1934) observed leucocytosis with shift to the left in patients with postencephalitic Parkinsonism, but like Economo (1929), Hoff (1938) and Beer (1942) we found no definite changes. We have examined two patients with Parkinsonism by sternal puncture, but did not find constant changes. Sato and Yoshimatsu

(1928), Shoji (1928), Lehmkuhl (1927), and Simmel (1931) noticed that the oxidase reaction became negative in chorea and in encephalitis Hoff (1932, 1938) and other workers could not confirm this. Diefendorf (1903), Hartmann (1906), Pappenheim (1907), Schrottenbach (1921) Wuth (1926) and Jackson *et al.* (1931), state that leucocytosis often occurs after paralytic attacks, while Elzholz (see Hoff, 1936) found it occurred also in delirium tremens and Sandri (1905), Schultz (1913), Zimmermann (1914) and Leupoldt (1928), Sagel (1931), Carrière (1931), and Jahn (1936) found it in schizophrenic exacerbations. Wittkower (1929) claims to have produced leucocytosis by hypnosis.

Although there is no solid basis for this theory, clinical observations and experimental research favour the existence of a central regulating mechanism governing leucocytosis. That such a central mechanism exists for the erythrocytes has been demonstrated by Sakurai (1933), da Rin and Costa (1934), Hayashida (1936), Dockhorn (1936), and Heilmeyer (1942).

Komiya (1938), in opposition to Beer (1942), denies the possibility of a direct relationship between midbrain and bone marrow, but admits a relationship between midbrain and liver. Stimuli are thus thought to be relayed from the liver to the marrow by humoral means. Hoff recognizes marrow hyperplasia induced humorally only following long-standing changes, such as chronic sepsis and diabetes. It is uncertain whether the ability of liver extracts to increase the number of leucocytes observed by Powers *et al* (1933), Foran *et al.* (1933), v. Bonsdorff (1934), Witts (1936), and Das Gupta (1939) has any connection with this phenomenon

Beer (1939) has studied such hormonal influences in the course of his research on the nervous-humoral regulation These will be discussed later He has proved that there are nervous connections from the vegetative centres to the hæmopoietic organs and to others which produce humoral factors These pathways descend in the spinal cord from the midbrain centres and emerge as sympathetic and parasympathetic nerves regulating blood flow. Parasympathetic fibres leave the spinal cord by the posterior roots and reach the bone marrow with the vessels and nerves The sympathetic fibres leave the cord by the anterior roots and *via* the communicating branches reach the sympathetic chain From there they reach the marrow with the peripheral nerves and vessels, some of them by way of the grey communicating branches Many experiments have been carried out to discover the influence of the vegetative nervous system on the blood picture In animals subjected to complete sympathectomy, Pasztor Lassak and Martin (1942), found a vagotonic blood picture with eosinophilia and lymphocytosis. When 50 million killed *B coli* were injected intravenously a leucocytosis and shift to the left occurred Beer

and Bedacht (1941) found that leucocytosis was not so severe in rabbits when the sympathetic chain had been cut. Asai (1940) applied electrical stimuli to the sympathetic system and found that more leucocytes than normal were released into the blood stream from the marrow. Okinaka *et al.* (1941) excised the parasympathetic system and observed that the number of leucocytes decreased and that there was then a tendency to fatty change in the marrow. When the sympathetic chain was excised, leucocytosis and marrow hyperplasia resulted, involving leucocytes, erythrocytes and platelets These results confirm Hoff's theory, that sympathetic dominance leads to a fatty marrow, but vagal dominance leads to a cellular marrow. Markoff (1942) agrees with this theory. He found that histamine increased bone marrow activity by acting *via* the vegetative nervous system He also alleges that it is chiefly the erythropoietic system that is affected. In a study of histamine asthma in the guinea pig, we found hyperplasia of granulocytes, and especially of eosinophils

Wakabayashi (1937) noted that leucocytosis followed vagotomy, indicating the dominance of the sympathetic system. The results obtained by Monteiro *et al.* (1933) tally with this study and so do the observations made by Bertelli, Falta and Schweiger (1910) that neutropenia, eosinophilia and lymphocytosis followed stimulation of the vagus. Hoff and Linhardt (1928) had previously demonstrated that in animals, after section of the cervical spinal cord bacterial injections failed to produce leucocytosis. Muto and Dohi (1935), found that this reactionary leucocytosis did occur when the cervical sympathetic chain was cut as well, but was abolished once more when the splanchnic nerves were cut on both sides Morikawa (1938) examined the marrow of tibia and femur Extirpation of the abdominal sympathetic chain resulted in a macroscopically demonstrable increase of the volume of bone marrow. The fat in the marrow decreased, and erythroblasts and megakaryocytes increased Section of the spinal parasympathetic fibres, on the other hand, produced atrophy of the red marrow and increase of the yellow marrow

Beer (1942) states that investigations with the aid of drugs are unsuitable when dealing with these problems But as no other method can reasonably be applied in human medicine, we have made blood and marrow examinations after stimulation and after inhibition of both the vagus and the sympathetic nerves by various drugs Our method of choice was the one devised in conjunction with Steinlin (1943) designed primarily for electrocardiographic examination, *e g*, stimulation of the vagus by acetylcholine, vagal inhibition by Bellafolin (Sandoz) stimulation of the sympathetic by adrenalin and Sympatol (para-methyl-amino-ethanol-phenol tartrate) and sympathetic inhibition by Gynergen (ergotamine tartrate). The changes found were mostly very slight, but they

helped us to understand the contradictory results of Okinaka *et al.* (1941) in the marrow and Monteiro *et al.* (1933) and Wakabayashi (1937) in the blood. Sympathetic stimulation produced a mature marrow and peripheral leucocytosis with a shift to the left whereas vagal stimulation produced a myelocytic shift to the left in the marrow and leucopenia in the blood. Rohr (1940) suggested that chronic stimulation of the marrow by sympathetic impulses led to a shift to the right in the marrow with predominance of segmented polymorphs, stab and juvenile forms (also including mature, orthochromatic normoblasts) and to a shift to the left in the blood, because stab forms and juvenile forms are ready to be passed into the blood stream. Our results confirm Rohr's theory. When, however, the marrow is inhibited by such influencing factors as over-activity of the spleen or the parasympathetic immature forms such as myelocytes and promyelocytes predominate in the marrow, and since these are not yet ready for release leucopenia results in the peripheral blood. At the same time early basophilic normoblasts may be seen in the marrow, the macrocytes in the blood in cases with anæmia, and also thrombocytopenia with giant platelets So far we have not seen platelet deficiency.

When the sympathetic dominates the picture other changes occur as well as leucocytosis the metabolic rate increases, protein becomes broken down, the blood sugar rises, acidosis sets in, the blood calcium rises, while the blood potassium falls (Wollheim, 1924; Zondek, 1927) and the blood cholesterol falls. Reverse changes are seen in vagotonia (Schilling, 1928, Hoff, 1938). Similarly, in diseases with a low calcium level, such as tetany, Hoff noticed low figures of leucocytes, and in diseases such as rickets, or osteitis fibrosa, accompanied by a rise in the calcium level, he found high figures Herzfeld, Lubowski and Kruger (1930) could not, however, demonstrate any definite relationship between the calcium level and leucocytosis. According to Barner (1927), Schilling (1928), Nielsen (1930), Gottsegen and Winkler (1933), Detre (1937) and Hoff (1938), leucocytosis with a shift to the left occurs in acidosis, and according to Steinmaurer (1932) an increase in platelets too Katase (1931) observed marrow hyperplasia in acidosis in animal experiments and this was confirmed by Markoff (1942) The collection of leucocytes in inflammatory foci is caused by local acidity (Schade and Mayr, 1930), which in turn favours movements of leucocytes by chemotaxis (Feringa, 1923, 1924, Jochims, 1927) The lowered surface tension (Schade and Mayr, 1930, Häbler and Weber, 1930), causes dilatation of the smaller vessels (Fleisch, 1921, Atzler and Lehmann, 1922), resulting in a slowing of the blood flow and producing collections of leucocytes at the periphery of the lesion. Hyperthermia, which enhances the amœboid movements of the leucocytes, may also play an important part (McCutcheon, 1923, 1924, Philipsborn, 1930) For the experiments

to demonstrate humoral agents the reader is referred to Beer's (1942) long paper. He was able to prove by extensive animal experiments that the only organ which is likely to produce such humoral agents is the liver.

Summary. Numerous factors may be responsible for leucocytosis and for leucopenia. Investigations by Hoff and Beer, and by other authors, especially the Japanese school, have apparently established the existence of a regulating mechanism in the tuber cinereum; the vegetative nervous system appears to act as the conducting pathway, possibly aided by a humoral agent.

THE STERNAL MARROW IN INFECTIOUS DISEASES

Schilling (1933) and his colleagues were the first to examine the sternal marrow in various infectious diseases. Yamamoto (1925) and Barta (1933) have made extensive studies. They described the following types of marrow reaction :—

Normal marrow: relatively acellular, mainly myelocytes, metamyelocytes, stab forms and segmented polymorphs.

Mature neutrophilic marrow with an increase of mature neutrophil cells from myelocytes to the mature segmented polymorphs.

Immature neutrophilic marrow with immature myelocytes.

Promyelocytic marrow of the relatively mature type with 70–80% promyelocytes of the more mature variety

Immature promyelocytic marrow with basophilic cells with round nuclei still showing myeloblastic features.

Myeloblastic marrow

Barta (1933) distinguishes :—

Moderate reaction with many mature cell forms.

Moderately severe reaction with increase of myelocytes and metamyelocytes.

Severe reaction with myelocytes and metamyelocytes.

Very severe reaction with promyelocytes

Inhibition of the marrow, with poor cellularity and many non-granular cells.

Just as in Yamamoto's (1925) animal experiments, Barta (1933) found that at the beginning of an infection (pneumonia) the marrow reacted with many mature cells, and later became still more cellular with myelocytes and metamyelocytes Contrary to Rohr's theory, Yamamoto observed a simultaneous shift to the left in blood and marrow in pneumonia. In septic diseases, Anselmino (1926) differentiated three types of reaction :—

Non-reactive, aplastic marrow

Increased reactivity,

(a) with an increase of leucocytes, chiefly myelocytic metamyelocytic and stab forms (myeloblasts only in severe cases),

(*b*) with an increase of monocytes.

Exhaustion of the marrow with an increase of reticulum cells.

The pioneer investigations of Schilling and his colleagues have been generally confirmed. The only point of dispute is whether myeloblasts participate in these reactions. Weiner and Kaznelson (1926), Nordenson (1935), Klima (1938) and Thaddea (1943) deny this but Schulten (1937) explains it on the ground that many investigators count the immature promyelocytes with the myeloblasts. Occasionally in rare cases the myeloblasts are increased, as we have seen in a case of typhoid fever. Out of 62 cases of infectious disease Nordenson noted only four in which the numbers of myelocytes and promyelocytes exceeded 30%, so that the shift to the left in the leucocytes is not often very great.

Rohr (1940) distinguishes three types :—

Mature stab form and leucocytic marrow.

Metamyelocytic—myelocytic marrow.

Mainly promyelocytic marrow.

Thaddea was unable to observe an increase of promyelocytes in the marrow in any case of neutrophil leucocytosis, but found such a marrow in monocytosis. He attributes the finding of promyelocytes by other workers to the misinterpretation of immature myelocytes. Lossen (1910) and Anselmino (1926) have seen a pronounced shift to the left more frequently in children, and Willi (1938) and Joppich and Lissens (1937) often found lymphatic hyperplasia. In latent infections the marrow may show changes, even if the blood picture is normal (Thaddea). Infections accompanied by leucopenia and those with leucocytosis cannot be differentiated on the basis of marrow findings (Leitner, 1941; Thaddea, 1943). With Klima, Stodtmeister (1938) and Thaddea, but disagreeing with Gloor (1929), Biernacki (1936), and Panà and Benvenuti (1937) we often found basophilia and toxic granulation of the neutrophil polymorphs. We believe these phenomena to be due to a disturbance of maturation in the marrow. Klima and Thaddea maintain that they correspond to the azurophil granulation. Thus the theory of the peripheral origin of toxic granulation put forward by Naegeli and Gloor would appear to be uncertain. By evaluation of Pontoni's maturation curves, Fieschi (1938) distinguishes the following types :—

(1) Normal curve in uncomplicated leucocytosis with a harmonious increase of evolution and proliferation.

(2) Shift of the curve to the left.

(*a*) with normal proliferation and inhibited evolution (inhibited type, *e.g.*, typhoid).

(*b*) with diminished proliferation and delayed evolution (*e.g.*, in commencing agranulocytosis).

(*c*) with increased proliferation of hæmocytoblasts (only in leukæmia and leukæmoid reactions).

(3) Shift to the right of the maturation curve in uncomplicated leucocytosis (inhibition of the release mechanism of the marrow).

Kienle (1943) states that a shift to the right of the maturation curve is a good sign since it indicates increased evolution and proliferation, which may also be shown by the presence of a large number of mitotic figures, especially the prophases. In toxic diseases a shift to the left is more often seen. Kienle believes that this is due to maturation arrest and not to increased proliferation. When the marrow was promyelocytic-myelocytic in type, Kienle found that the blood picture showed a marked shift to the left, but when it was promyelocytic-myeloblastic, leucopenia was quite frequently found. Proliferation and evolution do not always go hand in hand.

We believe that agranulocytosis, myelopathy and leukæmia should be regarded as completely distinct from the reactions due to infection. We suggest the following scheme for the classification of reactions of infection:—

Mature marrow with an increase of stab forms and metamyelocytes.

Metamyelocytic-myelocytic marrow.

Myelocytic marrow.

Myelocytic-promyelocytic marrow, in which the number of promyelocytes rarely exceeds 15%.

A completely unaltered marrow and, on the other hand, an increase of myeloblasts, should be regarded as extreme reactions, and are very infrequent in infectious diseases.

Klima (1938) observed qualitative changes of the white cells, such as basophilia, pyknosis of the nuclei, early lobation of the nuclei, enlargement of the whole cell producing "gigantocytes," loss of definition of the nuclear outline and a ground glass appearance; he also reported a reticulum cell reaction. Markoff (1937) also noted increase in the phagocytic reticulum cells in typhoid fever. Rohr (1940) reported increases of plasma cells and reticulum cells.

In our experience we have frequently seen an increase of plasma cells, but increases of reticulum cells have not been confirmed. The underlying disease process may change the marrow picture quite often in a specific manner, and in 1940–41 we began to classify these changes according to the disease rather than grouping them together under the general heading of infection.

Typhoid Fever

Galinowski (1938), from a careful study of 63 cases, distinguished 7 different types of reaction of granulocytopoiesis, and 8 types for erythropoiesis. Severe cases showed a shift to the left with up to 10% promyelocytes, but myeloblasts never exceeded 2·6%. With hyperplasia of the lymphatic organs the number of lymphocytes in

the marrow rose. The erythroblasts were increased in only 11·1% of the cases. Megakaryocytes remained normal.

According to Naegeli (1931) and Barta (1933) the marrow shows an increase of myeloblasts. We have observed this in one case. Storti and Fillippi (1937) and Fieschi (1940) report an increase of the granular series; in two cases Klima (1938) found a promyelocytic-myelocytic marrow, while Henning (1938) recorded a cellular marrow with a shift to the left, but Schulten (1937) found only a very slight shift to the left. Révol (1938) noted an initial increase of myeloblasts followed later by an increase of myelocytes, and also degenerative changes.

Fieschi found that mitotic figures in the myeloblasts were increased, but reduced in the myelocytes, and the number of prophases was high. Erythroblasts were only 7%–10% Like most of these authors, we have found eosinophilia in the marrow, even when the peripheral blood picture did not show it.

Paratyphoid Fever

In one case of paratyphoid-B septicæmia, Krummel and Stodtmeister (1936) observed a myeloblastic reaction in the marrow with myeloblasts in the peripheral blood But Schultz (1942) considered that this was actually a case of myeloblastic leukæmia in spite of the absence of extramedullary myeloid metaplasia. In a case of septicæmia from paratyphoid (type Schottmuller) organisms, we observed a promyelocytic-myelocytic reaction in the marrow:—

Case 44. H F, a man of 38 years, became ill with watery diarrhœa and fever up to 103° F after eating sausage When examined he was giddy and somewhat deaf. Agglutination tests for Salmonella Schottmuller were
were negativ
and later on

marked shift to the left, W B.C 6,080, basophils 1%, eosinophils 0%, stab forms 28%, segmented polymorphs 49%, lymphocytes 15%, monocytes 7%. Sedimentation rate 13–37 mm (Westergren 1 and 2 hr)

basophils 0·25%, eosinophil myelocytes 1·75%, eosinophil metamyelocytes 1·25%, eosinophils 2·25%, lymphocytes 8·5%, monocytes 1·5%, endothelial cells 3%, plasma cells 2·5%, megakaryocytes 2·5%, lymphoid and phagocytic reticulum cells 1%.

A marked shift to the left was present in the marrow (Fig. 167), while at the same time the blood picture showed a shift to the left with 28% stab forms.

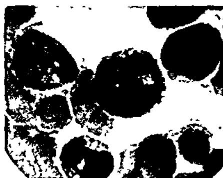


Fig. 167. Promyelocytic myelocytic marrow in paratyphoid-B-septicæmia. (×1,000)

Influenza

Schnetz and Greif (1937, 1938) examined 14 patients and found leucopenia in the blood and at the same time granulocytopenia in the marrow. Révol (1938) recorded slight eosinophilia. In our experience we have noted a slight myeloid shift to the left.

Typhus Fever

Tuschinsky and Kotlarenko (1932) observed many myeloblasts when the disease reached its peak, and subsequently many promyelocytes, an increased number of megakaryocytes, monocytosis and proliferation of the plasma cellular reticulum in the marrow. Schwenkenbecher (1943) in a series of 30 cases reported a marked state of irritation of the marrow, increased granulocytopoiesis with shift to the left, toxic granulation, decrease of eosinophil cells (followed by eosinophilia during convalescence), plasma cell proliferation and an increase of early basophilic normoblasts and orthochromatic normoblasts. In the guinea pig and also in man during the febrile period, Botzaris (1938) found a metamyelocytic shift to the left (up to a fivefold increase), and a decrease of eosinophils, followed by lymphocytosis and eosinophilia during convalescence

Pneumonia

Following the first observations of Schilling and his school, Klima (1938) reported 2 cases with a considerable increase of promyelocytes and myelocytes (13% and 28% promyelocytes and

30% and 26% myelocytes respectively) in the marrow. According to Barta (1933) and Nordenson (1935) the shift to the left of the granulocytes corresponds exactly to the severity of the infection, and this is also our experience. Révol (1938) found a myelocytic reaction and an increase of erythroblasts and hæmohistioblasts and toxic granulation in the granulocytes. He states that the latter is less marked in the marrow than in the blood. Like Thaddea (1943), we found that toxic granulation was more marked in marrow. Fieschi (1940) reports a promyelocytic marrow. In 2 cases of pneumonia with pulmonary abscesses, Aubertin and Morin (1938) observed a myelocytic shift to the left, hypoplasia of erythropoiesis and a decrease of eosinophils. In 17 cases of lobar pneumonia and 7 of bronchopneumonia, Bertola and Ravetta (1938) saw increases of promyelocytes and myelocytes, and hypoplasia of erythropoiesis. In 25 cases of chronic bronchitis and lung abscess they observed similar, but less marked, marrow changes. Eosinophilia was also present in cases of lung abscess. In cases of acute pulmonary abscesses, D'Agostino (1939) found increased erythropoiesis at the expense of the granulocytes; but in chronic cases there was hypoplasia of granulocytopoiesis at the expense of the red series. Our 2 cases of lobar pneumonia showed a promyelocytic-myelocytic shift to the left (11% promyelocytes, 22% myelocytes and 12·25% promyelocytes and 22·5% myelocytes respectively). One case of lung abscess showed a myelocytic shift to the left without erythroblastic hyperplasia. Thaddea recorded immature myelocytic marrow pictures Rohr, Révol, Henning and Keilhack (1939) noted reticulum cell hyperplasia. Treatment of cases of pneumonia with sulphonamides does not appear to influence the marrow (Bullowa *et al*, 1940, Forssell, 1942), or at most only slight disturbances of maturation have been seen (Barasciutti and Tondi, 1940)

Rubella

Moeschlin (1940) examined 21 patients and concluded that the myeloid cells merely show a slight shift to the left In contrast to the increase of plasma cells in the blood, these cells are not increased in the marrow.

Mumps

Révol (1938) found that the eosinophil and basophil cells were increased, and that there was a metamyelocytic shift to the left.

Chicken Pox

Rohr (1940) reported a myelocytic-metamyelocytic shift to the left. Révol (1938) found a slight increase of granulocytes We have observed a slight metamyelocytic shift to the left in the marrow in 3 cases.

Septicæmia

Klima (1938) found moderate shifts to the left in subacute bacterial endocarditis, septicæmia, pyæmia and gonococcal septicæmia In septicæmia, Plenge (1937) found the myelocytes increased up to 83%, while Fieschi (1940) reported an increase in the promyelocytes and Rohr (1940) in the plasma cells We have usually found merely a myelocytic shift to the left in cases of septicæmia such as the following case of subacute bacterial endocarditis :—

Case 45. M. F., a farmer aged 35, had not been well for 8 months. On examination: fever up to 102° F., enlarged soft spleen, systolic murmur over the mitral area, a trace of albumen in the urine Streptococcus viridans was recovered from the blood and by marrow culture.

BLOOD R B.C. 4 6 millions, Hb. 71 2% = 11·5 g %; W B.C. 11,000; eosinophils 3%, stab forms 10%, segmented polymorphs 64%, lymphocytes 18%, monocytes 5%. Sedimentation rate (Westergren) 86–120 mm (1 and 2 hr)

STERNAL MARROW. Proerythroblasts 0 75, early basophilic normoblasts 3 25, normoblasts 21 5 per 100 white cells; myeloblasts 1·75%, promyelocytes 4 75%, semimature myelocytes 5 75%, mature myelocytes 11·75%, metamyelocytes 13 25%, stab forms 11 25%, segmented polymorphs 19 5%, eosinophils 0%, lymphocytes 7·75%, monocytes 1·75%, endothelial cells 2%, megakaryocytes 0·75%, plasma cells 4 25%, primitive and phagocytic reticulum cells 2 25%

There was only a moderate shift to the left similar to that of Case 10 (p 119), which had streptococcus viridans septicæmia and anæmia, where 14·25% myelocytes and 16·25% metamyelocytes were found We have not seen leukæmoid reactions, such as were described by Poinso and Carcassone (1937) and Osvaldella (1932).

Tuberculosis

Degenerative changes were seen by Schilling (1933) and Yamamoto (1925) in post-mortem material and by Tempka and Braun (1932), Leitner (1935) and Klima (1938) in marrow biopsies. But Biernacki (1936), Panà and Benvenuti (1937), only found a metamyelocytic, and rarely a myelocytic, shift to the left, and no toxic changes Gottlieb (1931), Aubertin and Morin (1938) and Labendzinski (1938) reported a mature neutrophilic marrow with a tendency to transition into the immature stage, but never a promyelocytic marrow Labendziński failed to find an increase of monocytes in the marrow even in the presence of monocytosis in the blood, and this has been our own experience This favours the theory of their extramedullary origin The following case may serve as an example —

Case 46. S P, a tailor's apprentice aged 17 years, fell ill in June, 1943, with pain in the right side of the chest and fever up to 102° F Pleurisy with effusion was diagnosed On examination the whole of the right side of the chest was dull, and radiographs showed a dense shadow filling one

side almost completely. 500 ml. of serous exudate were withdrawn Tubercle bacilli were not found. The fluid contained mainly lymphocytes

Blood. R B C. 4·35 millions, Hb. 95% = 15·2 g.%; W.B.C 6,600; basophils 0·3%, eosinophils 2·3%, stab forms 4·3%, segmented polymorphs 46 6%, lymphocytes 18%, monocytes 27%, plasma cells 0 3%. Sedimentation rate (Westergren) 24–56 mm. (1 and 2 hr.).

Sternal Marrow. Proerythroblasts 1, early basophilic normoblast, 5 25, normoblasts 32·25 *per 100 white cells*; myeloblasts 0 75%, promyelocytes 1·75%, semimature myelocytes 4 25%, mature myelocytes 13·75%, metamyelocytes 21%, stab forms 31·5%, segmented polymorphs 10%, eosinophil myelocytes 2·25%, eosinophil metamyelocytes 3·25%, eosinophils
megakaryc
primitive
0·25%, endothelial cells 0·25%.

This patient showed a very high monocyte count in the blood (27%), but the numbers of monocytes or promyelocytes in the marrow were not increased.

Klima (1938) reported cases with a definite shift to the left. Quattrin and Filla (1940), in a series of 100 cases, found the hæmocytoblasts increased, a myelocytic-metamyelocytic shift to the left and a shift to the left of the erythroblasts, plasma cell proliferation and in several cases some immature lymphocytes. They classified their findings according to the various forms of tuberculosis Primary tuberculosis resulted in an increase of megakaryocytes and slowing of the development of the red and white cells. In the exudative types, erythropoiesis was inhibited, and in open tuberculosis not only was development of the red series delayed, but there was dissociation between the formation and maturation of the white series. They also found that the lung and marrow findings agreed very closely. In cases of early infiltration, Markoff (1936) noted slight increases of myelocytes and stab forms. Lanza (1930) reported a series of 28 cases He found appearances suggesting degeneration in the nuclei and in the cytoplasm of marrow cells, such as vacuolation, azurophilic and toxic granulation and dissociation of nuclear and cytoplasmic maturation When anæmia co-existed, erythropoiesis became slightly more dominant, when there was a toxic variety of tuberculosis, there were large erythroblasts, and following loss of blood there were micro-erythroblasts He found, as we did, plasma cell proliferation Like Bernabo-Silorata and Saita (1938) we observed only a moderate shift to the left in miliary tuberculosis, but Révol (1938) reported severe marrow reactions We have seen these only in the terminal stages preceding death László and Marton (1943) examined 102 patients by blood pictures and sternal puncture and found the latter much more reliable. They believe that a myeloid shift to the left indicates activity of the tuberculous process whilst hyperplasia of the reticulum suggests satisfactory powers of resistance. Trautwein (1944) recorded similar results. He also claimed to have established that

treatment, including chemotherapy, of tuberculosis resulted in a definite improvement of the marrow picture. Cremer and Gewecke (1943) rarely found a myeloid shift to the left in their cases. When the blood picture shifted to the left, the marrow, they state, shifts to the right. Cappellato and Chemasi (1942) examined the peripheral blood and sternal marrow in 37 patients from 1 to 60 hours after refills of an artificial pneumothorax, they did not notice any constant changes in the cell pictures. In pneumothorax cases they found eosinophil cells in excess in the exudate fluid, but as there was no eosinophilia in the marrow, they presumed that the eosinophils originated locally in the exudate. We (Leitner, 1935) have recorded our findings in 24 patients and the results are shown in Table 15 (p. 282).

Most commonly a myelocytic-metamyelocytic shift to the left was present. The myeloblasts were never, and the promyelocytes only rarely, affected and then only slightly. Apart from the leukæmoid and myelopathic reactions in tuberculosis of the lymphatic and hæmopoietic systems discussed in preceding sections, differentiation of the individual forms or stages of tuberculosis is quite impossible on the basis of the marrow findings. We cannot agree with László and Marton (1943) and Trautwein (1944), who consider sternal puncture a more reliable aid to the estimation of the progress and prognosis of tuberculosis than the blood picture. With our colleagues, Eichhorn and Vortisch, we have emphasized the value of the latter. The great variations in the figures for granulocytes in the myelogram make even moderately reliable conclusions impossible. All that can be said definitely is that the shift to the left is much more marked in severe tuberculosis than in slightly affected cases. In severe tuberculosis, toxic changes such as vacuolation, pathological granulation and dissociation of nuclear and cytoplasmic maturation may be seen frequently. We have not observed reticulum cell hyperplasia as a sign of adequate resistance. The most frequent reaction was plasma cell proliferation, which was present in slight as well as severe cases.

Boeck's Sarcoidosis (Morbus Besnier-Boeck-Schaumann, or Chronic Epithelioid Cell Reticulo-Endotheliosis)

It is still a matter of controversy whether this disease belongs to the tuberculosis group. Our recent findings (Leitner, 1945, 1946) favour its tuberculous ætiology. Dressler (1938), Esser (1940) and Gormsen (1940) found nodules of epithelioid cells in the sternal marrow, and it would, therefore, appear that sternal puncture is of diagnostic importance. We (Leitner, 1942) reported 15 cases, 8 of which were examined by sternal puncture, and have since investigated 13 cases, in 11 of which sternal puncture was carried out. We were not able to demonstrate nodules of epithelioid

TABLE

Boeck's

	1 L C	2 W B	3 F O	4 W R	5 B M
Blood					
R B C. in millions per cmm .	5 5	5 0	5 4	6 65	4 3
Hæmoglobin (100% = 16 g.%) .	91%	85%	93%	120%	88%
Leucocytes per cmm. . .	4,750	10,600	6,750	6,300	4,800
Basophil %	0 5	0 5	—	—	—
Eosinophil %	14 5	3 5	3 5	8 0	1·0
Stab forms %	0 5	3 5	2 5	1·0	4 0
Segmented polymorphs % . .	47 0	70 5	51·0	48 0	69 0
Lymphocytes % . . .	22 0	16 5	28 0	37 0	12 0
Monocytes %	15 5	5 5	15 0	7 0	14 0
Plasma cells %	—	—	—	—	—
Sternal Marrow					
White cells					
Myeloblasts %	1·3	1 0	2 0	1·0	0 3
Promyelocytes % . . .	5 6	5 3	4 6	3 3	3 3
Neutrophil semimature myelocytes % . .	14 3	6 6	7 3	5 3	10 6
Neutrophil mature myelocytes %	15 3	10 3	13 3	10 3	13 3
Neutrophil metamyelocytes % .	15 6	18 6	16 6	13 6	15 0
Neutrophil stab forms %	8 3	19 0	17 0	17 0	15 3
Neutrophil polymorphs % . .	8 0	12 3	10 0	16 3	20 6
Eosinophil myelocytes % .	3 3	3 0	2 3	5 0	1 3
Eosinophil metamyelocytes %	4 6	5 3	3 3	5 3	1 0
Eosinophils % . . .	6 3	2 3	3 3	3 6	1 6
Basophil myelocytes % . .	—	—	—	0 3	0 3
Basophils % . .	—	—	—	0 3	—
Lymphocytes % . . .	8 6	6 6	7 0	6 3	5 6
Monocytes % . .	1 3	1 0	1 3	1 6	1 0
Megakaryocytes % . .	1 6	1 0	2 0	1 6	2 6
Red cells (per 100 white cells)					
Proerythroblasts . . .	0 6	2 0	1 0	3 0	0 3
Early basophilic normoblasts	2 6	5 3	4 3	5 6	2 6
Late normoblasts . .	25 3	36 6	32 6	48 3	25 3
Reticulum cells					
Plasmoblasts % . .	0 3	0 3	0 3	0 3	0 6
Proplasmocytes % .	0 6	1 0	0 6	0 3	1 0
Plasma cells %	1 0	1 6	2 0	2 0	3 3
Lymphoid reticulum cells %	3 3	4 0	4 3	4 3	1 0
Phagocytic reticulum cells %	0 6	1 0	1 0	2 3	2 0
Endothelial cells % .	—	0 3	0 3	0 3	0 3
Fat cells %	0 3	-	—	0 6	-
Unidentified cells %	—	—	-	0 3	-

Sarcoidosis

6 K R	7 S M	8 N W	9 B R	10 K H	11 W M	12 J O	13 G E	14 M H
4 2	4 01	4 91	4 22	3 55	4 35	4 51	4 55	5 2
86%	80%	88%	92%	80%	94%	100%	92%	104%
5,400	8,000	4,800	5,500	11,550	6,700	3,200	4,450	4,000
—	1·5	1 0	0 5	2 0	—	—	—	0 5
4 0	8 0	5 5	3 5	5 0	3 0	5 0	2 5	2 5
7 0	2 0	1 5	0 5	10 0	5 0	—	2 0	3 5
51 5	40 0	53 0	63 5	66 0	50 0	65 0	67 5	52 5
24 5	46 0	30 0	10 5	14 0	21 0	27 0	20 5	28 5
12 5	2 5	9 0	12 5	3 0	12 0	3 0	7 5	11 5
0 5	—	—	—	—	—	—	—	—
0 6	0 3	0 3	1 0	0 75	0 6	0 5	1 0	1 0
3 6	1 3	1 6	3 75	2 25	2 3	1·75	5 25	4 5
6 6	3 6	6 3	5 25	2 5	6 3	6 75	8 25	3 75
22 6	10 3	19 3	12 25	18 25	14 6	11 25	23 0	9 75
14 0	20 0	28 3	13 5	22 25	16 3	20 25	19 0	15 0
14 6	19 6	20 6	16 75	22 0	20 0	19 5	8 75	19 75
10 3	26 3	17 3	18 0	16 5	15 6	17 0	12 25	19 25
3 3	6 3	1 6	5 25	0 5	2 0	4 0	3 75	5 0
3 3	5 0	1 0	7 25	0 5	0 6	4 25	3 5	1·5
2 0	5 3	1 3	4 0	0 5	1·3	3 25	2 75	1 5
0 3	0 3	0 3	0 25	0 25	0 3	0 25	—	—
0 3	0 3	—	0 25	0 25	0 3	0 25	—	—
10 3	7 6	4 3	3 5	3 5	8 3	4 5	7 5	6 0
2 3	1·3	0 3	0 75	0 5	1 0	1 0	0 5	2 0
0 6	0 3	1 6	1 0	1 0	3 6	1 5	0 5	1 0
1 3	0 3	0 6	1 0	1 0	0 3	0 5	1 5	1 0
2 0	0 6	7 0	1 25	2 5	2 3	2 0	3 5	4 5
18 6	18 0	34 3	18 75	25 25	25 3	30 0	24 0	31 75
0 3	—	0 3	—	—	—	—	—	0 [illegible]
—	0 3	—	0 5	0 25	0 3	0 25	0 25	0 5
1 0	0 3	1 6	2 0	0 75	1 3	1 75	0 5	4 0
6 6	1 3	1 6	1 25	1 0	2 3	1 0	0 75	3 0
1 3	0 3	0 6	0 75	0 25	1 0	0 5	0 5	0 75
—	—	—	—	0 25	0 3	—	0 5	—
—	0 3	0 3	—	—	0 3	—	—	—
—	—	—	—	—	—	—	—	—

cells in any, but in a few there was an increase of lymphoid reticulum cells. Santoianni (1936) and Lucia and Aggeler (1940) also failed to find any specific changes or epithelioid cell nodules in sternal marrow. When the bones are affected, as in osteitis fibrosa cystica, a positive result may be obtained by puncture if suitable sites are selected judiciously. Our results are shown in Table 16.

These results merely show a myeloid shift to the left, just as in other infectious diseases, and a slight increase of lymphoid reticulum cells in some cases. Two cases only showed remarkable marrow and blood pictures. One was a case of erythrocytosis with 6 65 million erythrocytes and 120% hæmoglobin. The other was one of thrombocythæmia with 875,000 platelets. The sternal marrow of the patient with erythrocytosis showed a definite increase in erythroblasts and the other a definite increase of megakaryocytes. The latter will be discussed in a subsequent chapter. These findings cannot be regarded as characteristic for epithelioid-celled granulomatosis, but are exceptional, especially the thrombocythæmia. Erythrocytosis has been recorded by other authors. It is usually considered to be a compensatory measure when the respiratory surface is much reduced owing to extensive pathological changes in the lungs. However, it is just possible that the erythrocytosis is a direct consequence of the involvement of the hæmopoietic organs, especially the spleen, in epithelioid-celled granulomatosis (Leitner, 1942). Stahel (1939) suggests that the myeloid shift to the left is a disturbance of maturation due to inhibition of the marrow by some splenic toxin. We believe that this applies only to cases with leucopenia and when the marrow is poorly cellular. Contrary to Stahel, we suspect a direct myelotoxic effect when the marrow is cellular.

Rheumatoid Arthritis

Mester (1938) collected 75 cases and found an increase of plasma and reticulum cells in acute and chronic rheumatoid arthritis Such changes were absent in degenerative joint disease Even when there was no increase of eosinophils in the blood, there was a marrow eosinophilia, but only in rheumatoid arthritis. The absence of this sign in other types of arthritis, especially in syphilis, suggested an allergic basis for rheumatoid arthritis. Debré, Millut and Lamy (1938) reported plasma cell proliferation In 55 cases Fleischhacker and Lachnit (1940) recorded a diminution of the number of marrow cells, and a slight increase of reticulum and plasma cells In Felty's Syndrome (leucopenia, splenomegaly, rheumatoid arthritis) they found a decrease of mature and an increase of primitive forms in the marrow In two cases of Felty's syndrome Ekelund (1943) noted an inhibition of the granuloblasts with a shift to the left. Buchler (1944) has reported a patient with Felty's syndrome in

whom leucopenia with marrow inhibition (promyelocytic marrow) was cured by splenectomy. Cattaneo and Cattaneo (1940) observed 11 cases of rheumatoid arthritis with hyperplasia of granulocytes and plasma cell proliferation and at the same time inhibition of erythropoiesis. In degenerative joint changes, Weitzmann (1941) found no abnormalities in the marrow picture. Amongst 44 cases of infective types of arthritis, the cases with positive bacteriological cultures showed a cellular marrow with reticulum cell proliferation, which was, however, not as marked as that recorded by other authors Eosinophilia in the peripheral blood was not always accompanied by marrow eosinophilia. One case with effusion in a joint was given amidopyrine and developed agranulocytosis. But even before that the total marrow count was as low as 17,000. In ankylosing spondylitis (Bechterew) when anæmia co-existed, there was hypoplasia of erythropoiesis and the eosinophils were increased. Révol (1938) obtained very variable results. In acute arthritis of infective origin, Greif (1937) found many myelocytes and promyelocytes, but in the chronic forms he found segmented and stab forms, the shift to the left never reaching beyond the metamyelocyte stage. In all our cases of rheumatoid arthritis we have observed plasma cell proliferation This, and the slight neutrophil shift to the left and eosinophilia, are points of importance in the diagnosis.

Case 47. G E., a labourer of 36 years, had had pulmonary tuberculosis since September, 1941. Sanatorium treatment improved his condition February, 1942, recurrence with high fever and cough. This was diagnosed as pneumonia and treated unsuccessfully with 24 grams of sulphathiazole. A short time before examination sore throat and joint pains When examined, there was bilateral, open pulmonary tuberculosis with cavitation, and left-sided thickening of the pleura. Elbow, hand and finger joints were painful, red and swollen One joint was aspirated and a clear exudate obtained Culture and animal inoculation proved negative

BLOOD. R B.C. 4 2 millions, Hb. 82% = 13·1 g %; W.B C. 9,200,

[illegible]

($0{\cdot}025\%$ $CaCl_2$)

STERNAL MARROW. Proerythroblasts 1, early basophilic normoblasts 3, normoblasts 22 6 per 100 white cells, myeloblasts 1%, promyelocytes 3 3%, semimature myelocytes 5 6%, mature myelocytes 11%, metamyelocytes 25 3%, stab forms 10 3%, segmented polymorphs 20 3%, eosinophil myelocytes 1%, eosinophil metamyelocytes 4%, eosinophils 3 3%, basophils 0 3%, lymphocytes 6 3%, monocytes 1%, m [illegible] roplasmocytes 0 3%, plasma ce [illegible] phagocytic reticulum cells 0 [illegible]

Here sternal puncture showed a metamyelocytic shift to the left, slight eosinophilia and plasma cell hyperplasia. The shift to the left was not so marked as in Greif's cases.

In chronic cases plasma cell hyperplasia may be even more pronounced.

Case 48. L. C, *a girl of 18 years*, had suffered from rheumatoid arthritis for the last four years. When examined, the joints of hand and feet were swollen and tender to pressure, and limitation of movements was very marked.

BLOOD. R B.C. 4·17 millions, Hb. 72·3% = 11·6 g.%; W.B.C.

9 46 g.%; albumen-globulin ratio 36 : 65.

STERNAL MARROW. Proerythroblasts 1, early basophilic normoblasts 1·5,

lymp

0·75

Both these cases showed an increase of serum protein with hyperglobulinæmia, and eosinophilia in the marrow, but not in the *peripheral blood. It is mere conjecture that there may be* some relationship between plasma cell proliferation and hyperglobulinæmia. A generalized hyperplasia of the reticulo-endothelial system is more probable.

Smallpox

Schretzenmayr (1938) found a marked myeloid reaction and reticulum cell proliferation.

Scarlet Fever

We invariably found a more or less pronounced myelocytic-*metamyelocytic shift* to the left and *eosinophilia*, but without disturbance of maturation and also plasma cell proliferation *The only other report in the literature is* by Chalier and Révol (1938), who also recorded an increase of myelocytes During convalescence *the mature neutrophils and eosinophils are increased*

Case 49. D E, a girl of 15 years, with scarlet fever.

BLOOD. R B.C 4·7 millions, Hb 92% = 14 7 g.%; W B C 9,760, basophils 0 5%, eosinophils 12%, segmented polymorphs 40%, lymphocytes 29·5%, monocytes 7%, plasma cells 2%.

STERNAL MARROW Early basophilic normoblasts 2 5, normoblasts *18 per 100 white cells*, myeloblasts 2·5%, promyelocytes 2 5%, semimature myelocytes 4%, mature myelocytes 10%, metamyelocytes 32 25%, stab forms 22·25%, segmented polymorphs 7·5%, eosinophil myelocytes 4·5%, eosinophil metamyelocytes 2·5%, eosinophils 3%, lymphocytes 11·25%, monocytes 1·5%, megakaryocytes 0 25%, plasmoblasts 0 5%, proplasmocytes 1·25%, plasma cells 1 5%

Measles

Schilling (1933) was the first to point out the "multi-coloured" blood picture with an increase of plasma cells. Subsequently this has been generally confirmed by Robert (1938), Leitner (1941) and others. We have usually found an increase of plasma cells in the blood and generally also in the marrow, apart from a metamyelocytic shift to the left. This finding favours the theory of the medullary development of at least some of the plasma cells. Révol (1938) also noted lymphocytosis and monocytosis.

Case 50. D. W., a man of 25 years with measles, when examined showed the typical rash as well as Koplik's spots, conjunctivitis, bronchitis and laryngitis.

Blood. R.B.C. 4·5 millions, Hb 105·8% = 16·9 g %; W.B.C. 4,400; basophils 0·5%, eosinophils 0%, stab forms 7%, segmented polymorphs 67·5%, lymphocytes 13%, monocytes 8·5%, plasma cells 3·5%. Sedimentation rate (Westergren) 11–26 mm. (1 and 2 hr.).

Sternal Marrow. Proerythroblasts 0·5, early basophilic normoblasts 2, normoblasts 14 per 100 white cells; myeloblasts 1·5%, promyelocytes 7%, semimature myelocytes 3%, mature myelocytes 15%, metamyelocytes 28·5%, stab forms 17·5%, segmented polymorphs 8·5%, eosinophil myelocytes 0·5%, eosinophil metamyelocytes 0·5%, eosinophils 0·5%, lymphocytes 8%, monocytes 1%, megakaryocytes 1%, plasmoblasts 1%, proplasmocytes 1%, plasma cells 5·5%.

Erysipelas

In erysipelas, Aubertin and Morin (1938) observed a more or less definite shift to the left. We have confirmed this in 3 cases. Thaddea (1943) invariably found an immature myelocytic marrow picture.

Case 51. G. R., a woman of 38 years, in 1921 had "catarrh of the lungs" and for a time received sanatorium treatment. At the time of examination she had bronchiectasis of the right lower lobe. She was admitted with florid erysipelas of the face with fever up to 102·5° F. Blood pressure 150/110. Electrocardiogram: myocardial degeneration with the P–R interval prolonged to 0·22 sec.

Blood. R.B.C. 3·98 millions, Hb 80% = 12·9 g %, W.B.C. 11,500, basophils 0·5%, juvenile forms 1%, stab forms 10%, segmented polymorphs 62·5%, lymphocytes 17%, monocytes 9%, sedimentation rate (Westergren) 37–57 mm. (1 and 2 hr.).

Sternal Marrow. Proerythroblasts 1, early basophilic normoblasts 1·3, normoblasts 30·3 per 100 white cells, myeloblasts 1%, promyelocytes 3·3%, semimature myelocytes 7·6%, mature myelocytes 18%, metamyelocytes 14·6, stab forms 8·3%, segmented polymorphs 23%, eosinophil myelocytes, 4%, eosinophil metamyelocytes 1·3%, eosinophils 0·6%, basophil myelocytes 0·3%, basophils 0·3%, lymphoblasts 0·3%, lymphocytes 8%, monocytes 2%, megakaryocytes 2%, plasmoblasts 0·3%, proplasmocytes 0·6%, plasma cells 2%, lymphoid reticulum cells 1%, phagocytic reticulum cells 2%.

Her progress was satisfactory. Sixteen grams of sulphathiazole resulted in the cure of the erysipelas in four and a half days.

In chronic cases plasma cell hyperplasia may be even more pronounced.

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BLOOD. R.B.C. 4·17 millions, Hb. 72·3% = 11·6 g.%; W.B.C. [illegible]

Blood cholesterol 105 mg.%, alkali reserve 49 vol. %, serum protein 9 46 g.%; albumen-globulin ratio 36 : 65.

STERNAL MARROW. Proerythroblasts 1, early basophilic normoblasts 1·5, normoblasts 24·5 per 100 white cells; myeloblasts 2%, promyelocytes 2%, semimature myelocytes 4 5%, mature myelocytes 11%, metamyelocytes 13%, stab forms 19 5%, segmented polymorphs 22%, eosinophil myelocytes 3%, eosinophil metamyelocytes 0 5%, eosinophils 3·5%, lymphocytes 6%, monocytes 0 5%, megakaryocytes 0 5%, plasmoblasts 0 75%, proplasmocytes 1·5%, plasma cells 3·75%, reticulum cells 1·5%.

Both these cases showed an increase of serum protein with hyperglobulinæmia, and eosinophilia in the marrow, but not in the peripheral blood. It is mere conjecture that there may be some relationship between plasma cell proliferation and hyperglobulinæmia. A generalized hyperplasia of the reticulo-endothelial system is more probable.

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BLOOD. R.B.C. 4·5 millions, Hb. 105·8% = 16·9 g.%; W.B.C. 4,400; basophils 0·5%, eosinophils 0%, stab forms 7%, segmented polymorphs 67·5%, lymphocytes 13%, monocytes 8·5%, plasma cells 3·5%. Sedimentation rate (Westergren) 11–26 mm. (1 and 2 hr.).

STERNAL MARROW. Proerythroblasts 0·5, early basophilic normoblasts 2, normoblasts 14 per 100 white cells, myeloblasts 1·5%, promyelocytes 7%, semimature myelocytes 3%, mature myelocytes 15%, metamyelocytes 28·5%, stab forms 17·5%, segmented polymorphs 8·5%, eosinophil myelocytes 0·5%, eosinophil metamyelocytes 0·5%, eosinophils 0·5%, lymphocytes 8%, monocytes 1%, megakaryocytes 1%, plasmoblasts 1%, proplasmocytes 1%, plasma cells 5·5%.

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STERNAL MARROW. Proerythroblasts 1, early basophilic normoblasts 1·3, normoblasts 30·3 per 100 white cells, myeloblasts 1%, promyelocytes 3·3%, semimature myelocytes 7·6%, mature myelocytes 18%, metamyelocytes 14·6, stab forms 8·3%, segmented polymorphs 23%, eosinophil myelocytes, 4%, eosinophil metamyelocytes 1·3%, eosinophils 0·6%, basophil myelocytes 0·3%, basophils 0·3%, lymphoblasts 0·3%, lymphocytes 8%, monocytes 2%, megakaryocytes 2%, plasmoblasts 0·3%, proplasmocytes 0·6%, plasma cells 2%, lymphoid reticulum cells 1%, phagocytic reticulum cells 2%.

Her progress was satisfactory. Sixteen grams of sulphathiazole resulted in the cure of the erysipelas in four and a half days.

Diphtheria

De Filippi (1939) found hyperplasia of the granulocytic system involving the primitive stages as far back as the hæmocytoblast, and plasma cell proliferation. *Fieschi (1940)* observed a promyelocytic marrow in 2 cases. Our own 2 cases showed a moderate shift to the left up to the mature myelocytes. Rohr (1940) examined the sternal marrow in 52 patients and observed a tendency to hyperplasia, a shift to the left and eosinophilia in the marrow. He also noted *toxic changes, the extent of which corresponded to the severity of the disease. This tallies with our findings.*

Undulant Fever

In one case Wohlwill (1932) observed nodules in marrow, spleen and lymph glands. Albertini and Lieberherr (1937) describe the marrow as normal; Schmid (1939) as myelocytic or promyelocytic in type in uncomplicated cases. One of his patients died of *thrombocytopenic purpura. Walthard (see Schmid, 1939), in one* case, found nodules and small foci of necrosis in the marrow, which was otherwise partly fibrous and partly myeloid with an increase in [illegible] Schmid also writes of a patient who was seen [illegible] granulocytosis occurred as a [illegible] *Willa (1943) made extensive* studies of 11 patients, who were examined by sternal puncture, and noted inhibition of myelopoiesis with a shift to the left in 1 case, and plasma cell proliferation of 14% in another They describe in detail a case complicated by slowly developing agranulocytosis. We *have examined a woman patient with granulocytopenia and 80%* lymphocytes in the blood whilst the marrow showed a myelocytic *shift to the left We believed that we were dealing with a splenic* inhibition of the marrow with slight disturbance of maturation. In Schmid's case, in spite of normal marrow giant cells, there was *severe damage to thrombocytopoiesis.* We should like to designate this as functional thrombomyelopathy Markoff (1936) reported a case of marrow inflammation (osteomyelitis from Brucella abortus) where the marrow was fibrous *Scaffidi and Molino (1941) found* that in human brucellosis an increase of the primitive forms in the marrow corresponded to neutropenia in the blood picture, and that erythropoiesis was relatively diminished The histiocytic reaction was increased. Sundberg and Spink (1947) found granulomatous *lesions with epithelioid cells by sternal puncture in 4 of the 19 cases* they examined.

In cases of Malta Fever, Cattaneo and Cattaneo (1940) found myeloid hyperplasia, in which the more mature cells predominated, and normal erythropoiesis.

Dengue Fever

This is a relatively benign exanthematous infectious disease, which lasts only about a week. Azzí and Magliano (1937) found that structural changes and various abnormalities did occur in the myeloid cells

Lymphogranuloma Inguinale

Gsell (1939), in one case, found a plasma cell count of 30·8%, hyperproteinæmia and hyperglobulinæmia. Nicolas and Favre (1922) noted that the granulomatous tissue was very rich in plasma cells. Gsell's finding supports the view of Ravaut *et al.* (1924), that we are dealing here with a generalized disease process. With Rohr (1940) we believe that the myelocytic reaction in Gsell's patient was against the diagnosis of multiple myelomatosis, where a marked increase of myelocytes does not usually occur.

Syphilis

Oria, Ramos and Tranchesi (1938) recorded a marrow with small celled infiltration and increase in lymphocytes and plasma cells In 8 patients with long-standing syphilis we found no abnormalities in the marrow, and Thaddea (1943) has confirmed this. Benedetti and Nuti (1942), in 28 syphilitic patients, found some maturation arrest of white and red cells This occurred mainly at the level of the promyelocyte and the polychromatic normoblast, respectively The blood picture showed hypochromic anæmia, polymorph leucocytosis, eosinophilia and monocytosis, and in the late stages, leucopenia

Leprosy

In the peripheral blood, Cerri (1942) found hypochromic anæmia, toxic granulation, vacuolation and shift to the left of the leucocytes, and atypical monocytes In the sternal marrow there was a myeloid shift to the left with toxic changes and vacuolation, plasma cell proliferation and the presence of monoblasts and monocytoid reticulum cells (the parent cells of the atypical monocytes in the blood)

Serum Sickness

Markoff (1936) and Gormsen and Heintzelmann (1941) observed a definite plasma cell proliferation in the marrow.

Relapsing Fever

Angelini (1937) observed an increase of the granular series especially during leucopenia following a crisis. During pyrexia

myeloblasts were decreased in number, but hæmocytoblasts myelocytes and metamyelocytes were increased.

Kala-Azar

The blood picture shows a leucopenia with a relative lymphocytosis, which may progress to agranulocytosis. There is also a shift to the left of the granulocytes and a reduction of eosinophils with not infrequently toxic changes. According to Redondo (1941) and Bartsocas (1939) the identification of the parasites by marrow biopsy is of great importance in the diagnosis Giraud and Gaubert (1937) and Malamos (1937) also stress the importance of sternal puncture in diagnosis. Chatterjee (1946) found from post-mortem examination of the femoral bone marrow that in the first stage of the disease a hyperplastic condition is found with numerous histiocytes, many containing parasites. In the second and third stages there is a progressive diminution in the number of cells in the marrow and some megaloblasts(?) and erythroblasts are seen. There is also marked proliferation of reticular fibrils which have special histological characteristics.

Gas Gangrene

Tanahasi (1938) examined the marrow from limbs removed by amputation and found the marrow was mainly trabecular and poorly cellular. In the marrow of patients in whom the disease took a favourable turn, he found large, round or ovoid, monocytoid cells. He therefore advised amputation only in those cases in which the sternal marrow was of the latter type.

GLANDULAR FEVER (INFECTIOUS MONONUCLEOSIS)

According to Glanzmann (1929), Lehndorff and Schwarz (1932, 1933), Downey and Stasney (1936), Leitner (1940), Stiefel (1943) and others, monocytic angina, lymphoid celled angina, infectious mononucleosis and many "lymphoid reactions" with enlarged glands, belong to this group of diseases We have defined the important points of cellular morphology in the peripheral blood as follows :—

Lymphoblasts.
Large lymphocytes.
Monocytoid lymphocytes
Lymphoid plasma cells and
Small lymphocytes.

At the onset of the disease the juvenile cells with basophilic cytoplasm are pathognomonic The serum protein is not increased, or only slightly. Moeschlin (1940) states that the lymphoid plasma cells are not concerned with the production of blood protein bodies,

unlike the medullary plasma cells. As regards further morphological peculiarities and serodiagnosis (Hanganatziu, 1924; Deicher, 1926; Paul and Bunnell, 1932; and Davidsohn, 1938), the reader is referred to our paper (Leitner, 1940). The diagnosis can usually be made from the blood picture. The frequently found plasma cells make differential diagnosis from lymphatic leukæmia possible, and the lymphoid cells differentiate it from myeloid leukæmia. Henning (1936), however, recorded a case which was diagnosed as paramyeloblastic leukæmia on the basis of the blood picture, but sternal puncture established the correct diagnosis. With de Vries (1938) and Schultz (1939), we consider that the monocytoid cells were not actual monocytes, but atypical lymphocytes. We have frequently seen genuine monocytes with a weakly positive oxidase reaction.

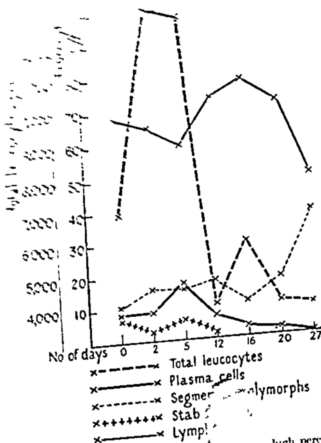
Most authors have not found lymphatic hyperplasia in the sternal marrow, amongst them Schulten (1937), Henning (1938), Klima (1938), de Weerdt (1939), Leitner (1940), Moeschlin (1940), Rohr (1940), Pinösch (1942), Thaddea (1943), Smith and Shaw (1945), Lamarzi *et al* (1946). Freeman (1936), Mirkoff (1936), Thomsen and Vimtrup (1939), Hateganu and Sparchez (1942) (in 4 of 10 cases) observed a lymphatic reaction analogous to the blood picture. Freeman's cases do not bear criticism. In the other cases the discrepancy of findings may be explained by the admixture of blood. Hyperplasia of the small lymph nodules in the marrow may also be considered as the cause of such odd findings, but such hyperplasia is usually very mild. Stiefel (1943) analysed the cases at the Zurich clinic and found normal figures of lymphocytes in only 9 in 29. The others showed lymphocytosis, but he admits that the admixture of blood is to blame largely for this result. There are very few cases where lymphocytosis may be attributed to hyperplasia of the marrow nodules. We have not seen such a case.

Case 52. M. E., a girl of 22 years, fell ill with a temperature of up to 101° F., malaise, depression, diarrhœa, and abdominal pain. When examined, she was pyrexial, had no sore throat, but there were some medium-sized, soft lymph glands in the neck.

BLOOD. R.B.C. 4·7 millions, Hb. 88% = 14·1 g.%, W.B.C. 6,900, basophils 1%, eosinophils 0·5%, stab forms 7%, segmented polymorphs 10·5%, lymphocytes 68%, monocytes 5%, plasma cells 8%. Two days later R.B.C. 4·0 millions, Hb. 78% = 12·5 g.%, W.B.C. 13,180, basophils 1%, eosinophils 2·5%, metamyelocytes 1%, stab forms 2%, segmented polymorphs 16%, lymphocytes 64%, monocytes 5%, plasma cells 8·5%.

STERNAL MARROW. Early basophilic normoblasts 0·5, normoblasts 17·5 per 100 white cells, myeloblasts 0·25%, promyelocytes 1·25%, semimature myelocytes 2%, mature myelocytes 13%, metamyelocytes 16·25%, stab forms 24·25%, segmented polymorphs 8·5%, eosinophil myelocytes 1·5%, eosinophil metamyelocytes 1%, eosinophils 4%, lymphoblasts 1%, monocytoid lymphocytes 10·5%, plasma cell-lymphocytes 3·5%, lymphocytes 7·5%, megakaryocytes 0·25%, plasmoblasts

... reticulum cells 0.5%. ... our counting was quite ... It is to be noted that the ... hyperplasia (Figs 168, 169). ... the progress are illustrated by



GRAPH 16 Glandular fever with leucocy... The ... high percen... lymphoid cells (more than 70%) ... was not at ... by lymph... lasia. (Figs. 16...

The juven... tic cells orig... lymph... Stahel (1939) ... (1940), ... es, ... able to demo... ly by pun... h gl... cases where ... diagn... (Lehndorff and ... 1933), or 1936), cannot ... from puncture is a ... of the oxidase rea... preparations with method has alrea... prevalent in the you...

Case 53. B. A., a nurse aged 31 years, with a past history of dry pleurisy. She fell ill with a sore throat and pains in the neck and when examined she had an inflamed throat, reddened tonsils covered with a



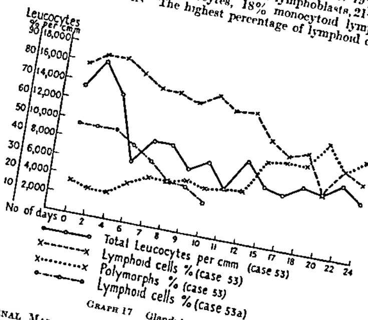
Fig 168 Neutrophilic marrow in glandular fever (× 500)



Fig 169 Neutrophilic marrow in glandular fever (× 500)

yellowish membrane-like material The regional lymph glands were enlarged.

Blood R B C 4 64 millions, Hb 100% = 16 g %, C I 1·07, W B C 12,500 (later 16,040), basophils 0·5%, eosinophils 0 5%, stab forms 2·5%, segmented polymorphs 14%, lymphatic cells 79%, monocytes 3 5%. Of the lymphatic cells, 2% were lymphoblasts, 21·5% large lymphocytes, 20% small lymphocytes, 18% monocytoid lymphocytes and 17·5% plasma cells The highest percentage of lymphoid cells was 83 5%.



Graph 17 Glandular Fever

Sternal Marrow Proerythroblasts 0 6, early basophilic normoblasts 3, normoblasts 31 4 per 100 white cells, myeloblasts 0 8%, promyelocytes 4·2%, semimature myelocytes 10 2%, mature myelocytes 18 4%, metamyelocytes 11·2%, stab forms 16 8%, segmented polymorphs

10·6%, eosinophil myelocytes 4%, eosinophil metamyelocytes 3·4%, eosinophils 3·6%, basophils 0·2%, *lymphoblasts* 0·2%, lymphocytes 5·6%, monocytes 2·2%, megakaryocytes 1·6%, plasmoblasts 0·4%, proplasmocytes 1·2%, plasma cells 2%, primitive reticulum cells 1·6%,



FIG. 170 Case 53 Sternal marrow · myelocytic marrow, absence of lymphoid cells (× 1,000)

In our series, the oldest patient (Case 53a, Graph 17) was a man of fifty-one years with open, bilateral pulmonary tuberculosis. We carried out a lymph gland puncture at the same time as the sternal puncture This showed lymphatic hyperplasia with juvenile lymphocytes and plasma cells. In none of our cases did the *sternal marrow show a figure* of lymphocytes higher than might be explained by the admixture of blood. We observed a myelocytic shift to the left (Fig. 170), which, *in Case 53, because the spleen* was enlarged, we believed was due to splenic inhibition of the marrow Stiefel (1943) regarded this myelocytic shift as due to infection Smith and Shaw (1945) and Limarzi *et al* (1946) in their cases found a marked shift to the left of the myeloid series with some maturation arrest In both Cases 53 and 53a the oxidase reaction was strongly positive in the marrow (Fig 171)

It is doubtful if the cases reported by Smith (1944) and Meyer (1946) belong to this group They described infectious lymphocytosis in upper respiratory infections in cases where the sternal marrow showed lymphocytosis, but there is reason to believe (Limarzi

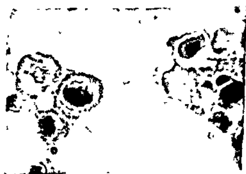


FIG 171 Oxidase reactions (according to Sato's method) in sternal marrow in glandular fever (× 1,000)

et al. 1946) that the lymphatic increase in the marrow was due to admixture of blood.

Summary. The sternal marrow does not as a rule show lymphatic hyperplasia in glandular fever. This is an important point in differential diagnosis.

In other infectious diseases the marrow findings are seldom characteristic, and rarely of value in diagnosis. Sternal puncture sometimes helps in assessing the severity and prognosis of the case. There is, however, no justification for exaggerating the value of sternal puncture. Findings within any one disease (for example, tuberculosis) are not sufficiently typical to define individual types of manifestations, though odd cases may fit well into certain schemes.

EOSINOPHILIA AND THE MARROW IN ALLERGIC DISEASE

The blood picture in allergic diseases is often typical, as stated r Schilling (1933), Stockinger (1933) and Leitner (1941). It is racterized by leucopenia with senile polymorphs, an inconstant inophilia and relative lymphocytosis. In 20 cases of allergy, zi (1940) found hyperplasia of the marrow with many early s of the leucocytic and erythroid series. Eosinophilia nore marked in the marrow than in the peripheral blood. ne histiocytes also took part in the hyperplasia. Habelmann (1940) observed an increase and a shift to the left of the eosinophils, an increased number of mitoses, vacuolation, plasma cell proliferation, a myeloid shift to the left as far as the promyelocytes, and a reaction of the marrow reticulum. Rohr (1940) states that there is a shift to the left in the marrow when the parasympathetic system is overactive. In our cases (Leitner, 1941) we have invariably found marked marrow eosinophilia when the blood showed eosinophilia. Initially, eosinophilia may be more pronounced in marrow than in blood. When the eosinophilic reaction subsided the number of eosinophils fell, first in the marrow and then in the blood. This is shown well in Case 55 (p. 301). Alexeieff (1933), Barta (1933), Gougerot and Dreyfus (1937) and we ourselves, have found that eosinophilia may be present in the marrow, even though it is absent from the blood. This may be conditioned by the time of examination as well as by a disturbance of the release mechanism of the marrow. The progress of marrow and blood eosinophilia is illustrated by the following cases in which sternal punctures were performed repeatedly:—

Case 54. A woman of 53 years with pernicious anæmia was treated with Campolon and during a course developed hypersensitivity against the liver preparation. As far as anæmia was concerned, the effect of Campolon was excellent, but the number of eosinophils rose from 1.5% to 34%

BLOOD (Dec. 19th) W.B.C. ...
stab forms 1·5%, segme...
monocytes 5%. Sternal m...
anæmia with megaloblasts...

BLOOD (Dec. 27th) ...
segmented polymorphs 58...

STERNAL MARROW. Pr...

myelocytes 8%, eosinophi...
0·25%, endothelial cells 0...
lymphoid an...

At that time the sternal ... 8·5% eosinophil meta-

This was a case of hypersensitivity against Campolon with marked eosinophilia. Cases following parenteral liver therapy have been reported by Strandell and Hammar (1931), Grün (1934), Criep (1938), Held and Goldbloom (1939), McSorley and Davidson (1944) and others. In Case 54, before the onset of eosinophilia in the blood, the sternal marrow showed normal numbers of eosinophils. At the first sternal puncture, performed during the eosinophilic period, the marrow showed 25·25% eosinophil cells (Fig. 172), while the blood contained 21%.

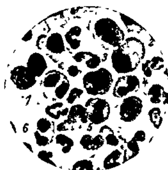


FIG 172 Eosinophilia in the sternal marrow. (× 500) The numbers mark the eosinophil cells

At the subsequent puncture the marrow contained 29·5% eosinophils, while the blood contained 34%. This would indicate that at the time of the first puncture the stimulus persisted and resulted in the further production of eosinophils and their release into the circulation. Correspondingly, the number of eosinophils in the blood rose. The lower figure of eosinophils in the next sternal marrow examination indicates that the power of the stimulus had decreased and eosinophil cells were released from the marrow. As a matter of fact, eosinophilia in the blood did decrease later, because no further excessive numbers of eosinophil cells were produced and replacement was not maintained at its previously high level. The liver preparation used was changed to Pernæmon forte, and the patient was given calcium In the first marrow biopsy we observed blue-grey granules in the mature eosinophils Similar marrow findings have been recorded by Stahel (1938), Leitner (1941), Nagel (1941) Loeffler and Maier (1943) in transient allergic pulmonary infiltrations

In our case the disturbance of maturation of the cytoplasm was particularly well shown by the grey-blue and multi-coloured granulations of the mature eosinophils (dissociation of nuclear and cytoplasmic maturation). The grey granules have been recognized already by Schleip and Alder (1936) as signs of a disturbance of maturation. Quite frequently we have also noted a shift to the left of the eosinophils. The following case of allergic infiltration in infestation with ascaris will serve as an illustration:—

Case 55. G. F., a girl of 20 years, whose mother had died of pulmonary tuberculosis, had been ill since March, 1940, with pulmonary tuberculosis. She had bilateral hæmatogeneous dissemination but no definite cavitation. On December 8th, 1941, she developed a large infiltration of the left lower zone and subfebrile temperatures. Blood examination on December 9th showed eosinophilia of 20.6%. Ova of ascaris were present in the stool. Following treatment with oil of chenopodium, she discharged fifteen worms. After that no more ova were seen in the fæces. The infiltration resolved completely in eight days. For blood pictures see Table 17.

TABLE 17
Case 55
Eosinophilia in Ascaris Infestation

	9.12	11.12	13.12	15.12	17.12	20.12	23.12	30.12
Leucocytes per cmm	13,550	10,250	8,400	10,200	5,950	4,950	9,400	7,150
Basophils %	1.0	0.3	0.6	0.5	—	2.5	—	—
Eosinophils %	20.6	23.0	15.3	7.5	6.5	8.5	5.5	2.0
Stab cells %	7.3	3.6	5.3	4.5	6.5	0.5	0.5	—
Segmented polymorphs %	50.0	41.3	51.3	61.5	62.5	62.5	65.0	59.0
Lymphocytes %	15.3	24.0	19.3	18.5	33.5	20.0	23.5	22.0
Monocytes %	5.3	7.6	7.6	7.0	9.5	8.0	5.5	17.0
Plasma cells %	0.3	—	—	0.5	—	—	—	—

STERNAL MARROW (December 9th, 1941). Early basophilic normoblasts 0.3, normoblasts 26.3 per 100 white cells, myeloblasts 0.3%, promyelocytes 2.3%, semimature myelocytes 2%, myelocytes 12%, metamyelocytes 6%, stab forms 16%, segmented polymorphs 27%, eosinophil myelocytes 19%, eosinophil metamyelocytes 8.6%, eosinophils 6.3%, lymphocytes 1.6%, monocytes 0.3%, megakaryocytes 1%, plasmoblasts 0.3%, plasma cells 0.6%, lymphoid reticulum cells 0.6%, phagocytic reticulum cells 0.3%, fat cells 0.6%.

We saw 34% eosinophils in the marrow, while in the blood the eosinophil count was 20.6%. Two days later eosinophilia in the blood increased to 23%. The previously definite shift to the left in the eosinophil cells (6% were juvenile forms) decreased. This might have been due to transmigration of the juvenile forms into the focus of infection, or possibly to a further partial maturation of these cells while in the blood stream. A few days later the transient infiltration was no longer recognizable, but the number of eosinophils in the blood was still 6.5%.

Nordenson (1936), Tottermann (1936), Vogel, Erf and

Rosenthal (1937), Harvier and Mallarmé (1939), Rohr (1940), Annoni (1941), Kienle (1943) and Thaddea (1943) have also reported eosinophilia in blood *and marrow, but the blood* and marrow findings do not always run parallel. Blood and marrow examinations suggest that the eosinophils develop in the marrow, and do not, as thought previously, originate at the site of inflammation. We have been able to demonstrate this phenomenon by parallel examinations of sternal marrow, blood and the contents of cantharides blisters. Working with Thalmann we found eosinophilia in blood, marrow and in the local focus of inflammation. The eosinophils appear to collect at the site of allergic inflammation, *e.g.*, the lungs (Leitner, 1941). The degree of eosinophilia in the blood depends largely on the local requirements at the site of the lesion.

Malmberg (1939), Atmar (1940), Cattaneo and Romano (1941) *have reported instances of familial eosinophilia.* The two latter authors examined 7 members of a family and found eosinophilia also in the marrow. There was, however, no disturbance of maturation. This is after all only natural, because there is no question of a sudden formation of an extra large number of eosinophils, but *the condition is chronic and well balanced.*

There was no maturational defect in the cases of *persistent eosinophilia with splenomegaly*, described by Brugsch (1931), Wieck (1931) and others. Meyer (1942) attributes the eosinophilia to a disturbance of the function of the spleen; Pöpping (1937) and Buchler (1940) to an allergic state; and Cremer (1939) to a reaction of the reticulo endothelial system. Buchler and Meyer found that the marrow showed mature eosinophils.

PATHOLOGICAL GRANULATION OF LEUCOCYTES

Gloor (1929), Biernacki (1936), Panà and Benvenuti (1937) believed that the so-called toxic granulation develops peripherally. Recent *observations of these granules in bone marrow*, by Klima (1938), Stodtmeister (1938), Leitner (1941), and Thaddea (1943), suggest that this is not the case, but that it is due to a disturbance of maturation. *The granules of the immature myelocytes appear to persist to a greater or lesser extent in the mature* myelocytes, owing to a dissociation of nuclear and cytoplasmic maturation. [illegible] to a disturbance of maturation. [illegible] curs together with basophilia, as [illegible] itner and Eichhorn (1932). Gloor (1929) and Barta (1930) believe the granules are due to the absorption of pathological products from inflammatory foci. Against this theory *is the fact that pathological granulation* may be seen in the myelocytes in the marrow, which have never been in contact with the inflammatory focus. Thaddea and Bakalos

(1940) think that they indicate an increase in the normal activity of the neutrophil cells. We believe, as does Stodtmeister, that they are of medullary origin. It is still not known why they are particularly common in certain infectious diseases, such as pneumonia (Gloor, Mommsen, Thaddea, Leitner), and tuberculosis (Leitner and Eichhorn)

THE HEREDITARY ANOMALIES OF LEUCOCYTES

Alder's Anomaly. This anomaly consists of a strongly basophilic granulation of the neutrophil and eosinophil cells. The latter in May-Grunwald-Giemsa preparations look rather like mast cells. Dr. Alder very kindly gave us some of his preparations, and by obtaining a positive oxidase reaction we succeeded in demonstrating the eosinophilic nature of the cells. The granulation of the neutrophils is distinguished from the "toxic" granulation by its intensity, and also because it occurs at a lower *p*H The pathological granulation is best seen at *p*H 5·4 The nucleus is rather poor in chromatin and we have ventured to suggest that Alder's anomaly may be due to a transfer of chromatin from the nucleus to the cytoplasm It is quite certain that disturbance of maturation of the cytoplasm plays a part, but normally such intense basophilia is not demonstrated even in the most primitive cells. Alder (1939) has reported strongly basophilic granulation of the cytoplasm in sternal marrow preparations also He considers that this peculiar anomaly in the blood is not merely an insignificant incidental finding, but must be regarded as an expression of a severe disturbance of health The two sisters examined by Alder developed considerable skeletal changes

FAMILIAL NUCLEAR ANOMALY OF LEUCOCYTES

Pelger-Huët Anomaly This anomaly was first described by the Dutch hæmatologist Pelger in 1928. It actually consists of inhibition of the normal segmentation of the leucocytes, simulating a shift to the left in the blood picture. Up to 30% or more of the leucocytes are stab forms, the others have two segments, cells with more segments are few and far between Usually a few metamyelocytes and occasional myelocytes are found. While the nuclear structure shows every sign of maturity, *i.e.*, it is compact, rich in chromatin and with coarse chromatin lumps, the nuclear form is of the juvenile type This "dissociation of formal and structural nuclear maturation" has been regarded as morphologically characteristic of the anomaly by Leitner (1938) and Leitner and Gugelot (1938) Investigations into the cause of this abnormality have not been pursued The oxidase reaction provides no evidence of disturbance of maturation

STERNAL MARROW. Proerythroblasts 1, early basophilic normoblasts 2, normoblasts 28·3 per 100 white cells; myeloblasts 1%, promyelocytes 5·6%, semimature myelocytes 10·6%, mature myelocytes 22%, metamyelocytes 18·6%, stab forms 13·3%, polymorphs with two segments 7·3%, eosinophil myelocytes 2%, eosinophil metamyelocytes 1·6%, eosinophils with two segments 2%, lymphocytes 7·3%, monocytes 1·3%, megakaryocytes 1%, plasma cells 3·6%, reticulum cells 2·6%.

The myelocytic shift to the left and the typical Pelger structure in the myelocytes was obvious and was present to some extent even in the promyelocytes. The megakaryocytes were less pleomorphic than usual. This patient's family, as well as full carriers, contained persons with a low grade dominance of the anomaly, and some members had leucocytes with three segments, but still with the typical Pelger form and structure of the segments. These lesser degrees of dominance are of importance if new cases are to be discovered.

Recently research into the anomaly has entered a new phase. Undritz (1939) and Tischendorf (1939) have demonstrated the anomaly in rabbits. Nachtsheim (1942, 1943) has established the dominant character of the anomaly in rabbits by the use of selective breeding. He was also successful in producing a pure strain of Pelger anomaly by mating a full carrier doe with a Pelger buck. This union produced only a few homozygous animals. Apparently some of the embryos were destroyed in utero. The surviving *homozygous rabbits made slow progress in growth and showed* manifestations of degeneration and severe skeletal changes. Undritz has examined these animals most carefully by serial blood pictures. He found leucocytes, all of which had round nuclei with senile structure, and some with advanced pyknosis. Because of the debility of the homozygous animals, marrow punctures were delayed. Undritz expected to find mainly cells with round nuclei. When eventually marrow puncture was performed, the findings were exactly as expected, viz., cells with round nuclei. The generalized degeneration and skeletal changes seem to indicate that the entire hæmopoietic reticulum of the Pelger animal is affected, or possibly even the mesenchyme. Partial carriage of the anomaly according to Nachtsheim's recent results cannot be recognized, because the genes are not divisible. Partial carriage could only be accepted if the hereditary progress is largely pleomorphic. The Pelger anomaly, however, with its regularly dominant hereditary character, is far from pleomorphic. The partial carriage, with segmented Pelger cells, described by us is called a "semitypical" Pelger anomaly by Nachtsheim. Terminology is, of course, not nearly as important as the fact that various degrees of dominance of the Pelger genes may be carried.

Nachtsheim's results clearly show that the Pelger gene is a pathological, undesirable one, the dissemination of which should be

avoided. The identity of the hæmatological pictures of the anomaly in the human and in animals leads us to suspect that we are dealing not so much with a harmless condition, but with a severe hæmopathy when it occurs in homozygous individuals. The anomaly is almost certainly more widespread than has been thought hitherto In conjunction with Leeuwen, and with Gugelot, we have examined 5 families with altogether 26 full carriers. The sternal marrow was characterized by a definite *myeloid shift to the left* and by a coarse chromatin structure in the granular cells

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ıkämie und die leukämoiden

S Karger, Berlin

the nature of an explosion, which caused splinters of the nuclei to break off also. Our own observations favour the development of platelets from the cytoplasm of megakaryocytes. Many authors, including Askanazy (1927), Naegeli (1931), Brugsch (1933) and Schulten (1939) hold similar views. Other observers, such as Petri (1925), found no evidence for this theory, but their findings were based on autopsy material. We reproduce here three typical and convincing photographs. Fig. 174 was obtained from the sternal marrow of a patient with nephritis. It shows a well preserved marrow giant cell with a well-defined nuclear structure, and close by there are some platelets. A short distance from the cell the photomicrograph shows a larger piece of cytoplasm and next to it another cluster of platelets. This is not likely to be a chance finding. Fig. 175 comes from the sternal marrow of a woman, aged forty-



Fig. 174.



Fig. 175

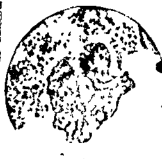


Fig. 176.

Fig. 174 Development of platelets from a megakaryocyte Platelets near the nucleus, further away a piece of protoplasm showing separation into platelets (×500)

Fig. 175 Development of platelets from a megakaryocyte (×1,000)

Fig. 176 Chance finding of platelets, close to a promegakaryocyte in whose nucleus nucleoli may be seen A small amount of basophilic, non agranular cytoplasm is sharply defined from the platelets (×1,000)

two years, who suffered from severe headache, possibly as the result of an industrial intoxication, since she did gilding work It shows a rather smaller megakaryocyte, the cytoplasm of which is separated into platelets. In both these cases erythropoiesis was normal. Fig 176 demonstrates the appearances of a chance picture in which a marrow giant cell is very close to a cluster of platelets It is a promegakaryocyte with a thin rim of well-defined basophilic cytoplasm without any granulation This is situated close to, but not connected with, a cluster of platelets The nucleus of the megakaryocyte is primitive and shows some nucleoli. Similar instructive pictures have been published by Willi (1935), Heilmeyer (1942) and others According to Endres and Herget (1929), the

platelets contain ions quite different from those of erythrocytes or of the blood plasma, and Vasaturo (1926) has shown that the iodine reaction of the granules of the platelets is identical with that of the megakaryocyte and with that cell only. The theory of their development from the megakaryocytes can thus be regarded as firmly established.

The object of marrow biopsy in disturbances of thrombopoiesis may be defined as the observation of changes of the marrow giant cells to provide guiding points for diagnosis, prognosis, and treatment. We divide the thrombomyelopathies as follows :—

Essential thrombocytopenia (Frank) or Werlhof's disease.
Symptomatic thrombocytopenia.
Hereditary hæmorrhagic thrombasthenia (Glanzmann)
Hereditary hæmorrhagic thrombopathy (Willebrand-Jurgens, Morawitz-Jurgens, Naegeli)

The marrow findings do not necessarily correspond to the blood findings. The myelogram is not usually characteristic for the various diseases of thrombocytopoiesis. In 1939 (see Leitner, 1944), we suggested the following scheme for the classification of the bone marrow findings in these disorders :—

(1) Aplastic reaction with disappearance of megakaryocytes (thrombomyelophthisis)

(2) Disturbances of the maturation of megakaryocytes, which we designate "thrombomyelopathy." The number of megakaryocytes may be normal or even increased There is a disturbance of maturation with a shift to the left which can be recognized morphologically. The immature megakaryoblasts and promegakaryocytes become dominant at the expense of the mature forms. There are also degenerative forms

(3) Thrombocytopenia or thrombopathy, which we call a "functional disturbance of maturation." The megakaryocytes in the marrow are normal and well granulated, but there is a deficiency of platelet-forming megakaryocytes. As there are few, or no platelets in the blood or in the marrow, we must presume that there is a disturbance of evolution.

(4) Peripheral thrombocytolysis in the spleen with normal platelet production In these cases megakaryocytes and platelets are present in the marrow in normal or increased numbers while there is peripheral thrombocytopenia

Type 2 is more common than type 3 amongst the disturbances of maturation The work of Glanzmann (1918), Lenggenhager (1936), Fonio and Schwendener (1942), Feissly (1943) and others has clearly demonstrated the part played by the platelets in the clotting of blood.

ESSENTIAL THROMBOCYTOPENIA (WERLHOF'S DISEASE)

In this form of thrombocytopenic purpura no underlying cause can be found. Its differentiation *from other similar states is not* possible on clinical and hæmatological grounds nor *on the basis* of sternal marrow biopsy. *According to Apitz (1943), there is,* as well as thrombocytopenia, another factor at work, because the remaining platelets do not *function as well as they should. Capillary* damage also is often present (Rosegger, 1938; Kibéd and Armentano, 1943). *Macfarlane (1941) and Bickel (1945) have shown* the importance of the vascular factor in the causation of hæmorrhagic diatheses. *As can be seen from our scheme of marrow reactions,* various authors have invoked various factors as the cause of thrombocytopenia. Frank (1925), *Catel (1937), Nickerson and* Sunderland (1937), Malamos (1940), Rohr (1940), Gonnermann (1941), Kienle (1942) and Leitner (1944), *have considered damage of the* marrow giant cells to be the cause; Frank (1925), Naegeli (1931), Fieschi and Villalobos (1939), Leitner (1944) *and others a* splenopathic disturbance of maturation with inhibition of platelet production, and Kaznelson (1916, 1919), Weiner and Kaznelson (1926), Fleischhacker (1937) and Rosegger (1938) an increased consumption or peripheral destruction of platelets. Nickerson and Sunderland state that the various forms of purpura cannot be *distinguished at autopsy. Rosegger, on the basis of marrow biopsy,* came to the same conclusion. In fatal cases of thrombocytopenia, Schmidt (1930) found normal figures of megakaryocytes, but Sternberg (1926) Jedlička and Altschuller (1926) and Dameshek and Miller (1946) report increases. We agree with Rohr, Schulten, Henning (1935), Markoff (1938) Heilmeyer (1942), and Thaddea (1943) that blood and marrow findings are not always strictly parallel. In marrow [illegible] Rohr, Fleischhacker [illegible] Heilmeyer, Thaddea [illegible] Mallarmé (1937), R[illegible] Markoff, Leitner, L[illegible] and Glanzmann (1945), found either normal or increased numbers of megakaryocytes. We have not found aplastic marrow reactions with an absence of marrow giant cells in Werlhof's disease, but have found that the number of megakaryocytes is usually increased, and we consider this a point in the differentiation from symptomatic forms of thrombocytopenia.

Morphological investigations often produce evidence of some disorder of function, *when counting the marrow giant cells fails to* help. Weiner and Kaznelson, Klima (1938), Weerdt (1939), Fieschi and Villalobos, *Thaddea, Glanzmann, and Dameshek* and Miller (1946) have found an increase of the immature forms at the

expense of the mature megakaryocytes. We have been able to confirm these observations. Rosegger (1938) recorded the following pathological changes: cloudy, ill-defined cytoplasm; lobed nuclei, and quite frequently also early forms. He does not, however, consider these changes to be of any importance in differentiating the various forms of thrombocytopenia. When the total number of marrow giant cells was increased, Seeliger (1924) noted degenerative changes, such as nuclear disruption, vacuolation, abnormal lobation of nuclei, and hyalinization of the cytoplasm. According to Limarzi and Schleicher (1940), the only disease in which hyalinization of the cytoplasm in megakaryocytes is seen is Werlhof's disease; and giant platelets are the result of this change. Rohr states that morphologically abnormal megakaryocytes occur frequently, showing immature nuclei, asynchronism of nuclear and cytoplasmic maturation, defects of granulation and giant forms. Fieschi and Villalobos (1938), in a series of 8 cases, found an increase in numbers and a shift to the left of the megakaryocytes The immature forms were increased: 7% megakaryoblasts, 78% granular forms (31 of which showed mottling), 11% of platelet-forming marrow giant cells and 4% merely nuclei (the number quoted for the granular forms appears to be normal according to our experience) Thaddea (1943) lays stress on the incomplete granulation of the megakaryocytes, vacuolation of the cytoplasm and clumping of the nuclei. Heilmeyer (1942) also recorded increased numbers of marrow giant cells, but the number of the platelet-

In this case the number of megakaryocytes increased tremendously soon after splenectomy It fell again after a fortnight, and the morphology of the megakaryocytes and the number of platelet-forming cells showed signs of returning to normal. Two weeks after splenectomy the number of platelets was increased, but blood and marrow were completely normal after three weeks

Abnormal function is not always associated with immature cells According to Schenker (1939), the immature forms may form platelets, especially when the degree of immaturity is not marked Kienle (1942) collected 12 cases, 3 acute and 9 chronic, in 8 cases the marrow showed a definite increase of marrow giant cells, and in 4 a doubtful one In several cases he found maturation arrest of the normal series with up to 11% immature forms with a basophilic cytoplasm, staining particularly deeply around the nucleus In 5 chronic cases he described a pathological developmental series, members of which were occasionally present in 4 other cases They were characterized by the histiocytoid, loose structure of their nuclei and by the clearness of the bluish-red cytoplasm, which contained few granules or none at

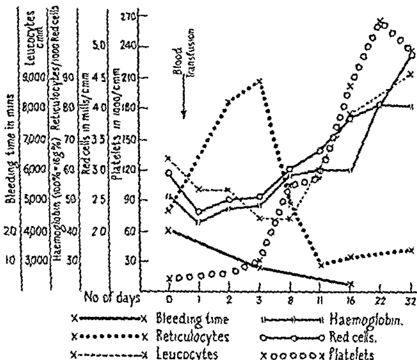
all. The basophilic nature of the cytoplasm is lost in the most immature forms, which show round or oval nuclei. A histiocytoid nuclear structure is still present in the mature cells. Kienle believed that this developmental series is an expression of faulty development, analogous to the megaloblastic series of pernicious anæmia, and possibly signifies a special disease within thrombocytopenic purpura in the wider sense. In a series of 8 cases, Jasinski (1944) found a shift to the left of the megakaryocytes and he quite justifiably considers the raised number of megakaryocytes as a natural consequence of the maturation arrest while the cells continue to be able to multiply. Dameshek and Miller (1946) found that in acute essential thrombocytopenia the megakaryocytes were increased from the normal maximum of 300 per million nucleated red cells to 366–743 per million. Platelet production was greatly diminished and was found in only 8%–19% of the megakaryocytes instead of the normal 68·6% Following splenectomy, platelet production was found in 69%–85% of the megakaryocytes. In chronic essential thrombocytopenia, similar results were obtained. Glanzmann (1945) obtained similar results.

It is certain that marrow biopsy enables us to recognize morphological characters and any abnormalities far better than was possible by post-mortem examination before the introduction of sternal puncture In our experience, megakaryoblasts normally number 0·5%–1%, promegakaryocytes 6·5%–8%, granular mature megakaryocytes 75%, and the number of degenerative forms with hyaline cytoplasm is normally about 15% Normally about ⅓–¼ of the granular forms belong to the platelet-forming variety.

Case 58. L M, a girl of 10 years, developed cellulitis of the right heel and osteomyelitis of the os calcis due to an ill-fitting shoe The localized abscess was incised in the surgical clinic and she felt much better On August 15th she broke a canine tooth by accident A few days later she developed bleeding into the skin and mucous membranes and also lymphocytosis in the peripheral blood With the diagnosis "acute lymphatic leukæmia or septicæmia" she was admitted to the medical clinic When
... over the chest, in the
..., and the wound
...e bleeding was
...ed, as estimated
by percussion, but was not palpable with certainty ... up to 100° F., pulse 160, extreme pallor, great apathy.

Blood. R B C 2·92 millions, Hb 51% = 8 2 g %. C I 0 85, W B C 6,380, basophils 0 5%, eosinophils 0 5%, metamyelocytes 0 5%, stab forms 5·0%, segmented polymorphs 47 5%, lymphocytes 41 5%, monocytes 4% *Platelets 11,600.* Bleeding time more than twenty minutes (Duke), clotting time 12 min 30 sec. (Burker) Clot retraction delayed Hess's test negative Reticulocytes 4 6% Anisocytosis, polychromasia Sedimentation rate (Westergren) 36–71–108 mm (1, 2 ... 2 hr)

mature myelocytes 12%, metamyelocytes 15%, stab forms 14·25%, segmented polymorphs 13·5%, eosinophil myelocytes 3%, eosinophil metamyelocytes 1·75%, eosinophils 3%, lymphocytes 6%, monocytes 1%, endothelial cells and lymphoid reticulum cells 4·[illegible] 3·5%, plasmoblasts 1[illegible] fat cells 0 25 [illegible] also of the r[illegible] cells was in [illegible] normal morphologically. There were [illegible] granular megakaryocytes, and the other 38% were promegakaryocytes. The latter had a basophilic cytoplasm with little or no granulation, but without any degenerative changes



GRAPH 18 Thrombocytopenic purpura with plentiful megakaryocytes in the sternal marrow, post hæmorrhagic anæmia (R.B.C. 2 92 millions Hb. 51% = 8 2 g %) and prolonged bleeding time Return to normal after blood transfusion, vitamin therapy, etc, with reticulocyte and platelet crises (giant platelets)

The patient was given an immediate blood transfusion, and a course of intravenous calcium and Redoxon injections (400 mg daily) was started immediately. She did very well Her blood findings are summarized in Graph 18.

In this case the anæmia appears to have been due to the hæmorrhages The co-existing osteomyelitis need hardly be considered as a causative factor, because it arose some time before the onset of purpura There is no evidence that the dental lesion was the primary one. The sternal marrow showed hyperplasia of all three systems, e g, of erythropoiesis, presumably owing to

anæmia, a slight shift to the left of granulocytopoiesis, and of the megakaryocytes, which were mostly mature, morphologically normal types (Figs. 177, 178). There was also an increase of lymphoid and plasma cellular reticulum cells.

Limarzi and Schleicher (1940) also found hyperplasia of the three principal cell systems, but were unable to offer any explanation. In our case the preceding infection may explain the shift to the left of granulocytopoiesis, and also the reaction of the reticulum, and anæmia will explain the erythroblastic hyperplasia. The increased number of megakaryocytes, the majority of them being morphologically normal cells, would indicate the type 3 of marrow



FIG 177.

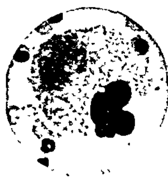


FIG. 178



FIG. 179

FIG 177 Sternal marrow in essential thrombocytopenia Megakaryocytes plentiful (× 150)

FIG. 178 Sternal marrow Same case Morphologically no sign of disturbance of maturation (× 1,000)

FIG. 179 Giant platelets in peripheral blood (platelet crisis) after blood transfusion (× 1,000)

reaction of our classification, i e, a functional disturbance of maturation. In spite of the raised number of megakaryocytes, we failed to find a single platelet-forming marrow giant cell in the examination of several slide preparations We have not been able to observe either in this or in 3 other cases of Werlhof's disease the phenomenon of giant platelets breaking away from megakaryocytes as described by Klima But during the remission, which set in after blood transfusion, we counted 25,800 platelets, many of them giant forms with basophilic hyalomere (Fig 179) With progressive improvement the platelet count reached 113,000, and the giant platelets disappeared The morphologically normal, non-platelet-forming marrow giant cells have been termed reserve cells by Frey (1928) They appear to form the majority of marrow giant cells in functional disturbance of maturation Probably our case

was one of medullary thrombocytopenia. The spleen could not be felt, but was increased in size by percussion. Nevertheless, there may have been some splenic inhibition of the marrow. Because of the controversial nature of this case and the availability of blocks of the photographs from previous publications, we have included it in this chapter, though it is not completely certain whether it is actually one of Werlhof's disease since there was a history of osteomyelitis two months previously. In 3 other cases, which proved to be essential thrombocytopenic purpura, we have made very similar observations on the marrows.

SYMPTOMATIC THROMBOCYTOPENIA

Drug Thrombocytopenia

Among the known causes of thrombocytopenia we find drugs, chemicals and compounds used in industry, and infections. Of the drugs, the derivatives of barbituric acid, Sedormid especially, occupy the most important place. Hadorn (1936), Lieberherr (1937), Gloor-Meyer (1937), and Falconer and Schumacher (1940) found low figures for megakaryocytes in Sedormid purpura. Hadorn found morphological damage, and Lieberherr vacuolation. It is interesting to note that amidopyrine damages the granulocytic system, and barbituric acid derivatives damage the marrow giant cell system. Since Dennig's first description of a case of drug purpura in 1933, numerous cases have been recorded. Falconer and Schumacher (1940), collected 42 cases from the literature, while Graeber (1942), apart from 3 of his own, collected 26 cases of Sedormid purpura According to Graeber, there is peripheral destruction of platelets as well as damage to the megakaryocytes In 4 out of 5 cases, Fleischhacker and Walterskirchen (1937) found normal figures for megakaryocytes; in the other case they were raised In 10 patients with Sedormid purpura, Moeschlin (1942) found only slight pathological changes in the megakaryocytes, consisting of inhibition of maturation and some normoblastic hyperplasia suggesting compensation for repeated loss of blood. Regeneration set in rapidly in 2–3 days, and in 5–7 days the platelets were normal in number once again. In one case Buchler (1944) found a normal marrow In a patient with Sedormid purpura we found the following marrow picture :—

Case 59. A man of 50 years, with repeated considerable hæmorrhages and anæmia

Blood Hb 60% = 9·6 g %, W B C 24,000, eosinophils 0·5%, metamyelocytes 0·5%, stab forms 5%, segmented polymorphs 80%, lymphocytes 7%, monocytes 7%, platelets 1,000 per cmm

Sternal Marrow Proerythroblasts 1·5, early basophilic normoblasts 3·75, normoblasts 47 per 100 white cells, myeloblasts 1%, promyelocytes 4·25%, neutrophil semimature myelocytes 6·75%, mature

[illegible] %, stab forms 15%, segmented 1·75%, eosinophil metamyelo-myelocytes 0 5%, basophils 0 5%, lymphoblasts 0·25%, lymphocytes 5·75%, monocytes 1·25%, megakaryocytes 3 25%, plasma cells 5 5%, primitive reticulum cells 1%, phagocytic reticulum cells 0 5%, fat cells 0 25%.

This case showed a slight increase of megakaryocytes (3·25%), with a shift to the left (1% megakaryoblasts, 2·25% more mature forms), an increase of erythroblasts (post-hæmorrhagic anæmia), and a myelocytic shift to the left The allergic reaction inhibits maturation, but *does not cause the megakaryocytes to disappear.*

There is some analogy with the agranulocytosis caused by pyramidon, where the drug is at first tolerated well and only later leads to sensitization. Markoff (1938), Meulengracht (1941) and Buchler (1944) were able to demonstrate the development of allergy experimentally. They induced marked reduction of platelets by giving small doses of Sedormid In similar experiments, Moeschlin *(1942) found that the platelets disappeared within* 60–70 minutes after the administration of the drug, suggesting a peripheral platelet destruction or sequestration rather than diminished production as a cause of the thrombocytopenia. Our own finding of the inhibition of maturation of marrow giant cells would suggest that platelet formation also suffers. Experimental transfusions of blood from patients with Sedormid purpura, carried out by Moeschlin, did not cause thrombocytopenia. This result tends strongly to exclude the presence of a thrombocytolysin. The allergic nature of Sedormid thrombocytopenia is also suggested by cases of purpura following the administration of Saridon, which contains only very *little* Sedormid *(Thiele, 1942)*

Maritschek and Markowicz (1933), Beiglböck (1937), Vogl (1938), Chapuis and Hemmeler (1944), have shown that quinine also may lead to thrombocytopenia In Chapuis and Hemmeler's case there was anaphylactic damage of two systems (agranulocytosis and thrombocytopenia) with eosinophilia. The first sternal puncture showed plentiful megakaryocytes with immature forms and the third puncture *revealed a commencing return to normal after the matur*ational disturbance The occurrence of agranulocytosis and thrombocytopenia together is very rare, except in allergic cases (Oettel and Thaddea, 1940)

Giauni (1941) reported a case of thrombocytopenia *after* 0 08 grams of bismuth, which recovered completely Sternal puncture showed hypersegmented marrow giant cells

Heinsen and Wachter (1942) observed a case of fatal thrombocytopenia in which only 0 3 g. of neo-salvarsan had been given The marrow showed complete absence of megakaryocytes The aplastic megakaryocytic reaction suggests toxic marrow damage The authors believe *that the purpura is due to the benzol ring,* because Kern (1938) and Mignolet (1939) described thrombocyto-

penia following benzol, and because Muller (1933) as well as Apitz and Huhn (1942) were able to reproduce it by giving this drug to animals.

A case described by Flood (1940) as thrombocytopenia due to sulphapyridine can hardly bear criticism, because it was complicated by open pulmonary tuberculosis

Sodium salicylate (Rappaport *et al*, 1945), mapharsen (Schwartz and Heide, 1945) neoarsphenamine (Hattersley, 1948), sulphathiazole (Strong and Glassburn, 1943; Donaldson and Scarborough, 1945), iodine (Dennig, 1933), gold preparations (Hatzky, 1932; Secher, 1939), Nirvanol (Jones and Jacobs, 1932), ergot (Peshkin and Miller, 1934), and insect bites (Fatzer, 1939) may lead to thrombocytopenia, some by toxic and some by allergic action. The marrow findings reported do not agree altogether.

From a review of the papers quoted and our own observations, it seems that in hypersensitivity reactions there is usually a normal or slightly decreased megakaryocyte count, the cells being morphologically normal (Rohr, Fleischhacker and Walterskirchen, Malamos, Vogl), or only slightly altered by a sort of shift to the left (Hadorn, Rohr, Gloor-Meyer, Lieberherr, Fatzer, Leitner, Giauni) According to Fleischhacker, the megakaryocytes are at first normal, later become increased in numbers and then show a tendency to a shift to the left. No conclusions should be drawn from a single sternal puncture in toxic marrow damage, aplastic marrow reactions with definite decrease of megakaryocytes (or even their disappearance) are more common Schwartz (1945) found the presence of high eosinophil counts in the marrow of value in distinguishing the allergic drug thrombocytopenias in which splenectomy was not indicated, from the essential thrombocytopenias in which splenectomy was required

Symptomatic Thrombocytopenia in Infectious Diseases

The thrombocytopenias in infections depend frequently on some agent toxic to the marrow, which may originate in the spleen especially when the underlying disease is accompanied by splenomegaly In cases of infectious thrombocytopenia, Kienle (1942) noted abnormally small megakaryocytes In our series, in the primary medullary forms we usually found an aplastic reaction with relatively few megakaryocytes, which were frequently degenerate. The following case will serve as an example —

Case 60. F. M, a woman of 20 years, with left-sided pulmonary tuberculosis with cavities and effusion, suddenly became ill, displaying a hæmorrhagic diathesis

Blood Thrombocytopenia of 5,000, bleeding time more than half-hour, clotting time eight minutes, clot retraction delayed Apart from a shift to the left the white cells showed no abnormalities

Sternal Marrow During the examination of several preparations no megakaryoblasts or megakaryocytes were seen There was also hypoplasia of erythropoiesis and a slight myelocytic shift to the left.

The patient succumbed a few weeks later. Blood transfusion produced only slight and transient improvement.

This case appears to be one of tuberculotoxic thrombomyelophthisis (type I of our classification), with splenomegaly. We have seen a second case, similar in all the main features, in a girl of eighteen years, with extensive pulmonary tuberculosis with cavitation and effusion and Raynaud's disease. She died of thrombocytopenic *purpura*. *Platelets* were *20,000* and *bleeding time* 28 minutes. In cases of advanced tuberculosis, Benedetto (1940) and Cutillo (1941) observed thrombocytopenic purpura. Not all thrombocytopenias are, of course, due to a primary marrow intoxication. Masure and Quérangal-Des Essarts (1937) recorded fatal thrombocytopenic purpura in tuberculosis of the liver and spleen, and Omodei-Zorini (1933), Natale (1934), Leitner (1940), Gerstenberg and Reinwein (1940), Weiner and Carter (1941) in tuberculosis of the spleen. Quite often, apart from the megakaryocytic system, other marrow systems become affected also. In our Case 38 (p 257), the thrombocytopenia, and for that matter myelopathy also, remitted completely after splenectomy, and the same thing happened in Omodei-Zorini's case Winternitz (1912) advised splenectomy in every case of tuberculosis of the spleen, because every case will end fatally unless operated on, but 59% recover following splenectomy

Sternal marrow *biopsy* is of great help in making the decision as to whether an operation is indicated or not Naegeli (1931) separated *lienal* thrombocytopenia with disturbance *of maturation* of the marrow giant cells from medullary thrombocytopenia with a decrease of marrow giant *cells*. *The first group in which* splenectomy may be expected to produce recovery comprises Werlhof's *disease and platelet deficiency states due to splenomegaly* (*e g*, Gaucher's disease, tuberculosis, Felty's syndrome). In marrow damage due to drugs, *blood transfusions are usually all that is* required According to Lawrence and Knutti (1934), Markoff *(1938) and others, splenectomy offers hope of success only in those* cases which show megakaryocytes in the marrow, but not in the *aplastic varieties* *Lawrence and Knutti further qualify* their statement and advise splenectomy only when megakaryocytes are *normal in numbers and appearances* As shown by Rohr (1940) Malamos (1940), Limarzi and Schleicher (1940), Heilmeyer (1942) and as *illustrated* by our Case 38 (p. 257), the blood and marrow pictures return to normal after splenectomy, and the *immature* or pathologically altered megakaryocytes are replaced by mature forms The importance of "splenopathic inhibition of the marrow" (Frank) or "hypersplenia" (Naegeli, Dameshek and Miller) has been proved by clinical and hæmatological studies, including biopsy examinations, and by experimental research

Bock and Frenzel (1938), in a case of portal obstruction, observed

inhibition of the marrow due to splenic disease, and attempted to reproduce the condition in experimental animals. They tied the splenic vein and the left gastric vein, so that the blood had to flow to the inferior vena cava *via* the left gastro-epiploic vein. Five animals developed normochromic anæmia, leucopenia and thrombocytopenia. The number of erythrocytes returned to normal in 10 weeks, but leucopenia and thrombocytopenia persisted much longer and disappeared only after 35 weeks with treatment by Nucleotrat. Jombres (1939) obtained similar results by tying the splenic vein. Bock and Frenzel maintain that the spleen produces substances which inhibit marrow function, but which are attenuated in the liver. The changes described only develop when this splenic agent acts on the marrow by passing the liver. Troland and Lee (1938) analysed the spleens of patients with thrombocytopenia and isolated from them a substance which caused a fall in platelets from 600,000 to 72,000 on injection into animals. They called the substance "thrombocytopen" It is claimed that its action lasts 24 hours. This work was confirmed by Rose and Boyer (1941) and Paul (1942), but not by Tocantins (1940) or Watson (1941) Moore (1940), however, relied on Troland and Lee's findings and on the fact that in the spleen of patients with thrombocytopenia the phagocytes are increased, to justify the theory of the peripheral destruction of platelets as the cause of thrombocytopenia. He regards the increase of megakaryocytes as merely secondary. Torrioli (1939) believes that the spleen produces a substance toxic to the megakaryocytes

Other infections, such as abortus fever, etc, may also lead to thrombocytopenia. We had occasion recently to observe an instructive case of septicæmia —

Case 61. B. H, a young woman of 21 years, who previously had been healthy, suddenly fell ill with high fever and hæmorrhages into the mucous membranes, epistaxis, bleeding from the bowel Blood pressure 112/70 mm. Hg

BLOOD R B C 3 1 millions, Hb 64% = 10 3 g %, W B C 2,300,
eosinoph
segmente
plasma c
17 000

cells showed marked toxic granulation, and vacuolation. Two more megakaryocytes were seen in further preparations, and they showed degenerative changes. Their nuclei were partly pyknotic and partly swollen, and their cytoplasm was hyaline and deficient in places.

Her progress was downhill The Hb *fell to 40% = 6·4 g.% in spite* of blood transfusions. The hæmorrhages did not cease. After a transfusion the platelets rose transiently to 154,000, only to fall again to 27,000, and the hæmoglobin fell to 22% = 3·6 g.%. Pyrexia continued Leucocytes remained at 3,500. *On the sixteenth day of the disease the patient* died The autopsy revealed generalized septicæmia.

Sternal puncture in this case showed hypoplasia of erythropoiesis, and *an infectious type of shift to the left of the white cells* with marked toxic changes, such as vacuolation, pyknosis, pathological granulation, as well as hypoplasia of megakaryocytes with degenerative changes of the scanty marrow giant cells, such as pyknosis, swelling of the nucleus with ill-defined contours, hyalinization of the cytoplasm and defects of granulation. Amongst 400 marrow cells counted we found one single megakaryocyte (0·25%), and in further preparations only two more marrow giant cells were seen. Thus the hypoplasia of the megakaryocytic system is actually greater than would appear from the result obtained from the count. Platelet-forming megakaryocytes were not seen at all This picture was obviously quite different from that seen in essential thrombocytopenia. *Sternal puncture, in a case of septicæmia from Strep. viridans*, showed 16,000 platelets and a similar marrow picture Kienle (1942) has also observed degenerative changes such as vacuolation and pyknosis in megakaryocytes in septicæmia.

Bock and Frenzel (1938) noted splenopathic inhibition of the marrow in portal obstruction. In cases of cholæmia with hæmorrhages, Kienle reported, quite apart from other causes, such as deficiency of Vitamin K, the frequent occurrence of thrombocytopenia with maturation arrest and with degenerative changes of the megakaryocytes in the marrow. These changes cleared up after operation and drainage *had removed the seat of the trouble*. Often extremely rapid platelet production followed the operation

In malignant disease, thrombocytopenia is not uncommon *It has been recorded by Kurpjuweit* (1903), Dünner (1921), Blum (1928), Schildknecht (1939) and Kienle (1942), and is also shown in our Case 20 (p 172). Blum found the marrow giant cells normal, Kienle found them damaged, Schildknecht could not find any at all. These changes are due to some factor toxic to the marrow *Kienle* also saw disintegration of the nucleus into fragments, and the cytoplasm showed similar changes Thrombocytopenia has also been described in cirrhosis *of the liver, Gaucher's* disease and other morbid states, and is often ascribed to splenic influence

Fowler (1940) assessed the incidence of symptomatic thrombocytopenia. *Of 160 cases*, 17 belonged to the essential and 143 to

the symptomatic form of the disease. He considers splenectomy useless in the latter, but with this we do not wholly agree. Splenectomy can have a very favourable effect in chronic forms of splenomegaly with thrombocytopenia (for example, in tuberculosis of the spleen). The beneficial effect of splenectomy in cases of essential thrombocytopenia with the corresponding changes in the blood and bone marrow have been described by Leitner (1944), Hemmeler (1945) and Dameshek and Miller (1946)

HEREDITARY HÆMORRHAGIC THROMBASTHENIA (GLANZMANN) AND CONSTITUTIONAL THROMBOPATHY (WILLEBRAND–JÜRGENS)

In a case of hereditary hæmorrhagic thrombasthenia, or Glanzmann's disease, Sapinski (1942) found that the megakaryocytes in the sternal marrow were normal. Functionally, therefore, they were presumably inferior to normal cells.

In cases of hereditary thrombopathy (Type Willebrand-Jurgens) Jurgens and Graupner (1937) observed vacuolation of the megakaryocytes and the production of agranular platelets Fleischhacker and Grüneis (1938) noticed small megakaryocytes, which apparently had lost the faculty to produce platelets. In the blood they saw large platelets, up to 11μ, which showed marked granulation Jelke (1942), however, found an increase of erythroblasts and of megakaryocytes.

Glanzmann (1945) has described important new observations on the bone marrow in thrombasthenia He found that the total number of marrow giant cells was slightly increased and described the presence of megakaryocytes with a nucleus, which was slightly lobed, and whose chromatin showed a peculiar check-pattern, had a wide meshwork, and was not dense Their cytoplasm was poor in granules, slightly basophilic and reddish brown In certain forms of thrombocytopenia Kienle (1942) observed similar cells Therefore Glanzmann presumed that such thrombocytopenias developed on an underlying thrombasthenia These observations of Glanzmann's provide us with a morphological basis for understanding the part played by platelet dysfunction in thrombocytopenia, which may well be more important than was thought hitherto.

Macfarlane (1941) and Perkins (1946) offer another explanation of the bleeding in constitutional thrombopathy. They found, by use of the capillary microscope, that there was a failure of normal capillary constriction following trauma, so that hæmorrhage was prolonged

OTHER HÆMORRHAGIC DIATHESES

Among the remaining *hæmorrhagic diatheses*, *scurvy* is the most important. Schulten (1939) in cases of scurvy found the figures for megakaryocytes normal, but erythropoiesis was hyperplastic owing to the loss of blood. McMillan and Inglis (1944) describe a normoblastic reaction in 4 cases and a megaloblastic reaction in one other, while Israëls (1943), on the other hand, found the marrow usually hypoplastic, the normoblasts being especially depressed. Mallarmé (1937) reported numerous mature megakaryocytes, which were sometimes not as granular as normal ones and showed some vacuoles Changes in the marrow giant cells can hardly be expected, because the primary damage is surely situated in the vascular field Anagnostu (1939) has observed damage of the megakaryocytes in the marrow and thrombocytopenia in the peripheral blood in animals which had been kept on a diet free of Vitamin A.

Not unnaturally it used to be thought that sternal puncture was contra-indicated in *hæmophilia*. Kočár (1943), however, maintains that it is harmless He observed an increase of megakaryocytes, especially the immature forms, and an increase of lymphocytes. In post-mortem material, Custer and Krumbhaar (1935) noted slight increases of marrow giant cells, but Révol (1938) found a normal marrow. Limarzi *et al* (1946), in 4 cases, found the megakaryocytes morphologically normal, though they appeared to have an accelerated rate of maturation and platelet formation There was hyperplasia of the myeloid and erythroid elements which could be correlated with the hæmorrhagic tendency of the disease Wright (1946) found the platelets in 4 cases to be significantly less sticky than normal.

In one case of *anaphylactoid purpura* (Henoch-Schönlein syndrome) we observed a normal marrow. Metz (1940) recorded a case of *hereditary hæmorrhagic telangiectasia* (Rendu-Osler-Weber disease) and reported that the differential diagnosis was made possible by the morphologically normal blood and marrow picture

Schönholzer (1939) examined a case of *fibrinogenopenia* by sternal puncture He found a slight metamyelocytic myelocytic shift to the left slightly reduced figures for megakaryocytes and normoblasts, an increase of the lymphoid and a decrease of the plasma cellular reticulum cells There may have been some connection between the latter finding and the lack of formation of fibrinogen Jürgens and Trautwein (1930) describe a case of *fibrinogenopenia* where the bone marrow was almost completely destroyed as the result of metastases from a prostatic carcinoma. Austen and Quastler (1945) found the bone marrow to be normal in a case of *idiopathic hypoprothrombinæmia*

In a case of *polyostotic fibrous dysplasia* (Jaffé-Lichtenstein

syndrome), Bamatter (1942) found normal platelet counts in the blood, but the prothrombin time was prolonged and there were few megakaryocytes in the sternal marrow. Clotting time was also prolonged, but bleeding time was normal.

Summary. Marrow biopsies have considerably advanced our knowledge of the hæmorrhagic diatheses. In essential thrombocytopenia (Werlhof's disease), figures for megakaryocytes are mostly normal or increased Primitive forms are relatively increased, at the expense of mature cells. It is possible that there is even a special developmental series of cells separate from the normal course of megakaryocytic maturation There are morphological cellular abnormalities, such as small amounts of granulation, and, most striking of all, a deficiency of megakaryocytes showing active platelet formation. The reserve cells are increased, and the platelet-forming cells suppressed. On the other hand, in cases of Werlhof's disease, aplastic reactions with absence of marrow giant cells have not been seen, nor are there signs of degeneration in the cells Among the symptomatic thrombocytopenias the allergic forms, from drugs or other hypersensitivity reactions, may produce a marrow picture similar to essential thrombocytopenia. Sometimes an increase in eosinophils is present. Other similar platelet deficiencies occur in relatively benign infections. The symptomatic thrombocytopenias in severe infections, malignant disease, severe hæmopathies, such as leukæmia or Hodgkin's disease, or in intoxications from such substances as benzol, are frequently characterized by an aplastic marrow The few remaining marrow giant cells usually show severe degenerative changes The latter group should be considered primarily as a marrow intoxication. In Werlhof's disease and the other thrombocytopenias, the spleen has a dominating role.

Marrow biopsy is a valuable aid to diagnosis, and it also provides important pointers for the estimation of prognosis The aplastic reactions, *i.e.*, type 1, warrant a much less favourable diagnosis than the cases with a normal megakaryocytic picture or merely with inhibition of maturation, *i.e.*, types 2 and 3 Sternal puncture is important when the question of splenectomy arises. No success can be expected from splenectomy in the cases with aplastic marrow, but only in those with a normal megakaryocytic marrow or with inhibition of maturation.

HYPERFUNCTION OF THROMBOCYTOPOIESIS

Primary and Secondary Thrombocythæmia

Elsewhere (Leitner, 1944) we have discussed the morbid states belonging to this group We distinguished "primary or essential" and "secondary or symptomatic" thrombocythæmia. Many

infectious diseases, especially tuberculosis, may be accompanied by increased platelet counts. Therefore we do not make a diagnosis of thrombocythæmia unless the case shows a threefold increase of normal platelet figures (using Fonio's method, we regard 250,000–300,000 platelets per cmm. as normal), and at the same time *an increase of megakaryocytes* in the marrow. Possibly the cases with normal megakaryocytic figures, in which the platelet-forming variety *materially outnumbers the reserve cells, should* also be grouped with the thrombocythæmias. Cases of polycythæmia or of hyperplasia of other cell systems will not be discussed here.

Rowlands and Vaizey (1938) recorded essential persisting thrombocythæmia without polycythæmia. One of their cases was anæmic, with 2·8 million R.B.C and 28% = 4 5 g.% Hb. In 1 case the number of platelets was 1·2, and in the other 1·8 millions. This disease is of practical importance, because thromboses developed owing to the high *platelet figures. Uotila (1938) described a case* with 5 million platelets and an increase of megakaryocyte figures in the sternal marrow. Akazaki and Hamaguchi (1939) report a case of thrombocythæmia with 2·77 million platelets. In post-mortem sections the bone marrow showed hyperplasia of marrow giant cells. The liver and spleen were also infiltrated with primitive giant cells (suggesting the possibility of megakaryocytic leukæmia, or some conditions allied to it) Reid (1940) observed *a woman of seventy-one years. Her platelet count* varied between 1·73 and 3 8 millions, the leucocytes were 14–18,000, and erythrocytes 4·76–5·05 millions. The sternal marrow showed marked increases of megakaryocytes and of platelets, the bleeding time was prolonged in spite of thrombocythæmia, but the clotting time, *prothrombin time and clot retraction were all normal* Reid states that this is a chronic disease, mainly affecting older people, and that there is a tendency to hæmorrhages in the skin and mucous membranes, and to thromboses. His case developed *thrombosis in one leg. According to Reid, there is always a leuco*cytosis with a shift to the left, occasionally also eosinophilia and monocytosis, and in the sternal marrow hyperplasia of all three cell systems *is the rule.* These observations make it doubtful if *Reid's* case was *a true case of primary thrombocythæmia.* It may have been an atypical form of polycythæmia Petrescu, Olaru and Vereanu (1941) reported a case with 1·4 million platelets in which the diagnosis was either thrombocythæmia or megakaryocytic leukæmia Because there were only 2% giant cells in the marrow *it is not* likely that it was a case of megakaryocytic leukæmia

In our series we have had 4 cases of thrombocythæmia occurring in various diseases Three of them also showed hyperplasia of the megakaryocytes in *the marrow They have been described* in detail elsewhere (Leitner, 1944) One patient with generalized caseating tuberculosis of the lymphatico-hæmopoietic system had

a platelet count of 913,000 and at the same time anæmia, and normal or only slightly increased number of leucocytes. The number of megakaryocytes in the marrow was not increased. Tendency to bleeding was very marked, probably due to a deficiency of prothrombin from tuberculosis of the liver. The other cases occurred in patients with a carcinoma of the bronchus with metastases in the marrow, with Boeck's sarcoidosis, and with hyperadrenalism respectively.

Case 62. L. F., a baker, aged 26 years, suddenly fell ill with high fever, feeling dazed and disorientated and complained of stabbing pain in the splenic region, and vomiting. He was at first thought to be a malingerer, but later, enlargement of the liver and of the hilar lymph

blasts 6·3, normoblasts 40·6 per 100 white cells; myeloblasts 1·3%, promyelocytes 3·6%, semimature myelocytes 7·6%, mature myelocytes 11·3%, metamyelocytes 13·3%, stab forms 14·3%, segmented polymorphs 14%, eosinophil myelocytes 2·6%, eosinophil metamyelocytes

in weight. Sedimentation rate became normal. Anæmia remitted and the thrombocythæmia became less marked.

This was a case of Boeck's sarcoidosis with enlargement of the hilar glands, showing at the same time thrombocythæmia, anæmia and leucopenia. The number of marrow giant cells was 11·3%, the platelet count in the blood was 875,000. The profound general disturbance at the onset, together with severe pain in the splenic area, made it probable that thrombosis of the splenic vein had occurred. In all our 3 cases with secondary thrombocythæmia there was an isolated reaction of the megakaryocytic system, the erythropoietic and leucopoietic systems being unaffected. Occasionally there was slight or moderate anæmia. Thrombocythæmia was transient and no residual trouble remained, once it had subsided. There was no evidence of any direct connection with the underlying diseases, and in one case there was even osteosclerosis of the long bones. Cases with normal platelet counts do not belong to this group, not even when the megakaryocytes are increased in the marrow. This applies to Inden's (1938) cases. In 20 patients with *beri-beri* he found increases of the megakaryocytes without thrombocythæmia. In our patients with secondary thrombo-

cythæmia, the megakaryocytes were morphologically normal (Fig. 180). The number of the platelet-forming giant cells was definitely increased in absolute numbers, but relatively there was only a slight shift in favour of the platelet-forming megakaryocytes at the expense of the reserve cells.



FIG 180 Sternal marrow in thrombocythæmia Increase of megakaryocytes (× 600)

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CHAPTER XI

SUMMARY OF DISORDERS OF MATURATION

Among the many important findings which may be obtained by marrow biopsy, the disturbances of maturation of individual or of several cell systems are the ones which are of greatest interest, and about which we have learnt most by sternal puncture. They are, therefore, summarized in the following Table 18.

TABLE 18

Disorders of Maturation

Disorder	Type of disturbance of maturation	Characteristics		Ætiology
		In the marrow	In blood	
Pernicious anæmia	Disturbed maturation of nuclei of erythroblasts, early hæmoglobinization, inhibition of loss of nuclei. "*Dissociation* of nuclear cytoplasmic maturation"	Promegaloblasts, megaloblasts, giant forms of *neutrophils*, hypersegmented giant neutrophils.	Hyperchromic anæmia, megalocytes, inferior types of erythrocytes (poikilocytes).	Absence of anti-pernicious anæmia principle, Castle ferment, or disturbances of absorption of liver principle (*Sprue*); *toxic* influences (pregnancy, dibothriocephalus).
Agranulocytosis	1. Maturation arrest at the level of promyelocytes and immature myelocytes	1. Immature promyelocytic—myelocytic marrow.	1. Granulocytopenia or merely neutropenia.	1. *Idiopathic* or drugs, industrial intoxications, infections, pregnancy.
	2. Maturation arrest at the level of metamyelocytes, or stab forms; functional disturbance of maturation Disturbance of release mechanism in the marrow	2 Slight myeloid (metamyelocytic) shift to the left	2. Granulocytopenia	2. Ditto.
Panmyelophthisis anæmia	Maturation arrest of myelopoiesis (promyelocytic marrow), erythropoiesis (faulty loss of nuclei or hypoplasia), and thrombocytopoiesis	Promyelocytic marrow; hypoplasia of erythropoiesis (delayed evolution), immature and degenerate marrow giant cells.	Anæmia, leucopenia (granulocytopenia), thrombocytopenia (hæmorrhagic aleukia, aplastic anæmia).	As in agranulocytosis.

Thrombocytopenia	1 Disturbance of maturation morphologically recognizable	1 Predominance of immature and degenerate megakaryocytes	1. Thrombocytopenia.	1. Idiopathic or as in agranulocytosis
	2 Functional disturbance of maturation, diminution of platelet forming megakaryocytes	2. Predominance of non platelet forming, morphologically intact reserve cells	2. Thrombocytopenia.	2. Ditto.
Thrombopathy	As in thrombocytopenia 1 and 2	Degenerate or small megakaryocytes.	Thrombopathy, pathological forms of platelets.	Familial
Eosinophilia of sudden onset	Disturbance of maturation of the cytoplasm, dissociation of nuclear and cytoplasmic maturation, inversely as in pernicious anæmia; immature cytoplasm, mature nucleus (blue granules).	Immature blue-grey granules even in eosinophils with mature nuclei	Eosinophils with immature granules	Reaction of hypersensitivity.
Pathological granulation of neutrophils	Inhibition of maturation of neutrophilic granulation Segmented polymorphs with granulation of immature myelocytes	Increased granulation and basophilia of neutrophil myelocytes, metamyelocytes and polymorphs	Increased granulation of neutrophils	Infections, intoxications
Pelger-Huët's familial nuclear anomaly	"Dissociation of form and structure of nuclear maturation"	Coarse lumps of chromatin in the nuclei of promyelocytes and the more mature stages	Immature form, inhibition of segmentation, mature nuclear structure.	Familial, dominant hereditary factor

CHAPTER XII

DISORDERS OF THE MARROW RETICULUM

RETICULO-ENDOTHELIOSIS AND RETICULUM CELL SARCOMA

THE problem of the reticuloses bristles with controversy, and our understanding of it is still far from clear, though it has been studied very closely, especially during the last few years. Some of the reticuloses, as may be seen from Table 9 (p. 201), are closely allied to the leukæmias, and it might be argued that they should have been discussed in relation to that subject. Other forms of reticulosis, however, have no connection whatever with the leukæmias, and we therefore prefer to attempt a new classification from the clinical and pathological points of view.

Leukæmic and Aleukæmic Reticulo-endotheliosis

These have already been mentioned in the section on monocytic leukæmia. They must be separated from the other reticuloses, because in actual fact they are diseases of the reticulum of a leukæmic nature Fieschi (1942) published such a case in great detail. According to the morphological findings, he distinguishes various forms of leukæmic reticulosis, which he identifies with di Guglielmo's *histioleukæmia* :

(1) Reticulo-endotheliosis with primitive reticulo-endothelial cells

(2) Reticulo-endotheliosis with lymphatic cells (di Guglielmo).

(3) Reticulo-endotheliosis with monocytoid cells.

The last variant corresponds to the typical reticular monocytic leukæmia, the type Schilling of American writers. Fieschi's case showed *anæmia* (Hb 23% = 3 7g%), thrombocytopenia and leucocytosis (W.B.C 34,000), and 92 per cent of the white cells were histioblasts showing no monocytic characteristics The sternal marrow was very cellular and almost all the cells were of the same histioblastic type. They were 30μ in diameter and had a primitive, lobed nucleus, with two nucleoli. Autopsy revealed reticulosis with tremendous proliferation in the bone marrow, liver, spleen and lymph glands, and the cells were not so much fibril-forming or histioblastic, but rather blood-forming or hæmopoietic. Garin (1943) believes that there is a type of leukæmic reticulo-endotheliosis, which is not identical with monocytic leukæmia. He described two cases, with an acute course, accompanied by anæmia, thrombocytopenia and leucocytosis (up to 35,000) in which 56% of the

white cells in the blood a ... cells. One of the cases came t ... proliferation in the bone marrow, ... w were reticulum ... owed reticulum cell ... n and lymph glands. Boidin, Bousser and Delzant (1943) reported a case with very large cells with lobed nuclei These cells were found in the peripheral blood, the marrow, liver, spleen and lymph glands The nuclear structure was coarsely trabecular and spongy. The authors excluded reticulum cell sarcoma, because there was no infiltrating growth and no metastases Hueber and Velasco (1943) reported a case which they believed to be a reticulosis which showed anæmia, leukopenia and thrombocytopenia. In the sternal marrow the phagocytic reticulum cells were increased, and a tumour was found in the upper part of the abdomen Autopsy was not performed, and it is therefore uncertain whether this case was, in fact, one of leukæmic reticulo-endotheliosis

Apart from these acute "histioleukæmias" there are acute and chronic cases of clear-cut monocytic leukæmia. Thus pathological picture has been closely studied and described by many authors The references are given in the section on monocytic leukæmia (pp. 222–225) These cases are not a variant of myeloid leukæmia, i e, the so-called Naegeli type of monocytic leukæmia, but show proliferation of the reticulo-endothelial system in all the hæmopoietic organs. Some cases are entirely aleukæmic, in others monocytes may be numerous in the blood stream The combination of myeloid leukæmia and reticulosis has been described by Tronchetti (1939), but the classification of such a case is uncertain

Chronic Reticuloses. Other chronic reticuloses are allied to monocytic leukæmia, but there is no justification in assuming that they are identical They take a slow course without fever, but with slight anæmia, lymphocytosis (30 to 40 per cent) and monocytosis Böhne and Huismans (1932) described such a case. Wuhrmann (1944), Uehlinger (1930), Alessio (1933) and Penzold (1937) noted a generalized proliferation of the reticulo-endothelial system in the spleen, liver and lymph glands in histological sections Tschistowitsch and Bykowa (1928), Böhne and Huismans (1932), Roulet (1930, 1932) and Benecke (1940) found such proliferation in bone marrow also. It is probable that chronic infections play a part in its ætiology We have observed the following case —

Case 63. B F, a woman of 31 years, complained of lassitude, loss of appetite and a feeling of weakness, which had persisted for some time A mass in the mediastinum was discovered and she received treatment with X-rays, which resulted in a reduction in the size of the mediastinal shadow The symptoms continued and she was therefore admitted to the medical clinic When examined she had obviously lost weight The spleen was enlarged and hard, reaching almost down to the symphysis The liver was hard, and its edge was four fingers' breadth below the costal margin

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The enormous splenomegaly, hepatomegaly, enlargement of mediastinal lymph glands and the reticulum cell proliferation in the sternal marrow made the diagnosis of reticulosis the most probable one, but unfortunately an autopsy could not be obtained.

Acute Infectious Reticulosis (Abt-Letterer-Siwe's Disease)

This disease runs a course similar to that of septicæmia or sometimes of acute leukæmia. It is accompanied by a necrotizing angina, otitis, hæmorrhagic diathesis, and enlargement of the spleen, liver, and lymph glands. It has been discussed by Akiba (1926), Krahn (1926), Guizetti (1931), Podvinec and Terplan (1931), Gittins and Hawksley (1933), Siwe (1933), Foot and Olcott (1934), Roussy and Oberling (1934), Creveld and Ter Poorten (1935), Abt and Denenholz (1936), Glanzmann and Walthard (1940), Vahlquist (1942). Some degree of anæmia has been found in nearly all the cases of Letterer, Guizetti, Podvinec and Terplan, Creveld and Ter Poorten, Abt and Denenholz. Sometimes this was profound and progressive, as seen by Guizetti, Siwe, Gittins, Glanzmann and Walthard In most cases a moderate leucocytosis was seen, but monocytosis only infrequently, *e g*, Letterer and Siwe (10%), Guizetti, Roussy and Oberling (9%) The course is usually acute. Podvinec and Terplan's case survived only for 2 weeks, Guizetti's case 3 weeks, and Akiba's case 6 weeks. The only patients to live for more than a year from the onset were those of Foot and Olcott (18 months), Gittins (2 years), and Glanzmann (2 years 5 months). The disease attacks children exclusively and is invariably fatal. Pathologically there was generalized proliferation of the reticulum in the bone marrow, in liver, spleen and lymph glands, and sometimes in the lungs (Creveld and Ter Poorten, Glanzmann and Walthard) Other organs, such as the skin, tonsils and alimentary canal, have been affected in some cases The cases described by Ighenti (1931), Attig (1932), Merritt and Page (1933) and Glanzmann and Walthard (1940) were originally diagnosed as Hand-Schuller-Christian's disease. Attig and Ighenti fo... transitional stages between the still "empty" reticulum ... those laden with cholesterol Merritt an... e found li... e cells in some organs, and in

others only proliferation of the reticulo-endothelial system, which was, however, of an extreme degree. Glanzmann considers that Abt-Siwe-Letterer's disease is merely a syndrome and not a disease in its own right. He regards it as an extremely acute form of Hand-Schuller-Christian's disease in which, owing to the short duration of the morbid process, lipoid is not deposited in excess. In his case, as in that of Galeotti-Flori and Parenti (1937), the close connection between reticulo-endotheliosis and lipoid granulomatosis was well illustrated Galeotti-Flori and Parenti examined material from the hæmopoietic centres in the spleen, lymph glands and bone marrow and found reticulum proliferation without evidence of lipoid storage. When the case came to autopsy, however, they found a typical lipoid granulomatosis The lipoid granulomatoses will be discussed later (pp 353–356).

Atypical cases of Hodgkin's disease, such as those of Schultz, Wermbter and Puhl (1924) and Sachs and Wohlwill (1927), are at least allied to the reticulo-endothelioses, in the opinion of Doan and Wiseman (1934), Cionini and Rotta (1934) and Hittmair (1942) Penzold believes that they should not be regarded as identical conditions The case described by Schultz, Wermbter and Puhl is still a subject of controversy, and thought by many to belong to the reticuloses Malignant lymphadenoma, or Hodgkin's disease, will be discussed in a separate section (pp 357–362), after the reticuloses, as both conditions have many points in common

Miscellaneous Types of Reticulosis

There are cases of reticulosis in adults which show neither leukæmic characteristics nor a definite relationship to infections or to diseases of storage Kienle (1943), observed a patient with marked pyrexia, bone pains, swelling of the inguinal glands, but without abnormal findings in the blood The sternal marrow was very cellular, with large cells with a basophilic, sometimes greenish cytoplasm and fine nuclear structure with numerous nucleoli There were also "giant cells" with nuclei divided into two or three lobes Histological sections showed groups of these cells in the liver and spleen, but at these sites the proliferation was not nearly so intense as in the bone marrow. Kienle excluded leukæmic disease on these grounds Bertola (1940) reported a similar case It is possible that these cases are infectious reactions of the reticulum in response to an unknown pathogenic agent

Proliferation of the reticulum may occur in other infectious diseases It is quite probable that most are reticuloses conditioned by an infection We have termed Boeck's sarcoidosis "chronic epithelioid-celled reticulo-endotheliosis or granulomatosis," because many organs, especially the spleen, liver, lymph glands

and bone marrow, show a purely epithelioid-celled granulomatosis. We presume that under the influence of infection the cells of the reticulo-endothelial system and of the mesenchyme generally, become transformed into epithelioid cells in the widest sense of the word. Dressler (1938), Stahel (1939) and Esser (1940) found epithelioid-celled granulomata in the sternal marrow. Such a finding, however, is not the rule and in our series of 19 cases (Leitner, 1940) examined by sternal puncture, we have not seen it. Santoianni (1936) and Leitner (1940), often found a slight increase of the primitive reticulum cells in the sternal marrow, but not invariably, and this is not constant enough to be of value in diagnosis. Stahel and Leitner *suggest puncture of the glands as a procedure* more likely to yield information. Gebsattel (1920), Mylius and Schurmann (1929), Uehlinger (1930), Berblinger (1939) and others invariably found a generalized epithelioid-celled proliferation in this disease and this would seem to justify our conception of it. Certain cases fail to provide clinical evidence of a generalized *reticulo-endotheliosis*. This, of course, is due to the relatively benign nature of the disease, which tends to undergo spontaneous cure before all the organs containing reticulo-endothelial cells are affected to any obvious degree. In most cases there are more organs affected than can be recognized clinically. Various organs may be affected at different times, and a definite statement on the full extent of the disease can only be given after several years of observation.

Many authors, such as Janbon and Favre (1942), Taillens (1943) and others regard infectious mononucleosis as a reticulo-endotheliosis. The necessary pathological basis for such a conception is not yet available. In other infectious diseases reticulum cell proliferation has been observed in the sternal marrow. Santoianni (1936), Tzanck, Dreyfuss and Levy (1938), and De Weerdt (1939) found increased numbers of reticulum cells in mycosis fungoides. Tzanck counted up to 30%, De Weerdt in 4 cases up to 20%, and in 5 cases he found only a slight increase of reticulum cells. This is a *secondary reaction* of the reticulum and has been called "associated reticulosis." Oberling and Guérin (1934) suggested this term for the reactionary proliferation of the reticulo-endothelial system in agranulocytosis and the panmyelopathies when these showed hypoplastic marrow.

Marrow biopsies have been carried out only *infrequently* in the various *reticuloses*. A number of cases have been mentioned already. Arinkin (1929) reported a case with more than 80% reticulum cells in the sternal marrow. Dameshek (1933) found an increase of monocytes, marrow giant cells and histiocytes, Fieschi (1942) observed histiocytes, Rohr and Hegglin (1936), pleomorphic cells with lymphoid structure, which Schulten (1937) thought might have been *identical* with the cells seen by Arinkin,

and Dameshek; similar cells have been observed by Weber and Huber (1939). Patrassi and Crepet (1939) described megaloblasts and other cells resembling megaloblasts. This is probably due to a difference in nomenclature. In the sternal marrow, Hueber and Velasco (1943) found phagocytic reticulum cells; Fieschi (1942) histioblasts; Kienle (1943) and Bertola (1940) large basophilic cells with many nucleoli, Garin (1943) saw an increase of reticulum cells; Boidin, Bousser and Delzant (1943) reported large cells with lobulated nuclei, and we have found (Leitner, 1940) an increase of primitive reticulum cells.

Well-defined, tumour-like proliferations of the reticulo-endothelial system have been described by Wihman (1931), Roulet (1932), Oberling and Guérin (1934), Dawson, Innes and Harvey (1937), Cathala and Boulenger (1941), Heilmeyer (1942) and others. They are mentioned here because they cannot with certainty be differentiated from the reticuloses. According to Rössle (1939) there are forms intermediate between reticulosis and reticulum cell sarcoma. His colleague, Roulet (1932), had published an excellent paper on the latter. These proliferations do not consist of any specific tissue. The proliferations of the reticulo-endothelial system of the lymph glands consist of cells, which are not the parent cells of lymphocytes, but of the monocytes capable of phagocytosis and the production of fibrils. Rössle and Roulet distinguish three forms: an immature cellular form, a more mature fibril-forming one, and a mixed cell form. The tumours arising from reticulum cells are intermediate from the nosological point of view between leukæmia and the more common tumours of connective tissue. Cathala and Boulenger (1941) do not think that it is possible to make a distinction between the reticuloses and reticulum cell sarcoma. Lubbers (1942) described a case which he called "leukæmic polyblastic retotheliosis". On the basis of a biopsy of the cervical gland tumour the diagnosis of retothelsarcoma was made. Autopsy and histological section, however, showed reticulo-endotheliosis characterized by reticulum cell proliferation from the Kupffer cells in the liver, and from cells in the adventitia in the psoas muscle. Roulet (1930, 1932), Loesch (1932) Ungar (1933), Downey and Stasney (1936), Apitz (1940), Ahlström (1933, 1941), and Heilmeyer (1942) have described similar cases.

According to Cathala and Boulenger (1941) the reticuloses are characterized by leucopenia, monocytosis and splenomegaly. They state the leucopenia is a point against there being any connection with leukæmia, but since leukæmia is commonly aleukæmic, this argument would not appear to carry much weight. In their case the sternal marrow showed a proliferation of the reticulo-endothelial system. The cells were of the size of the large lymphocytes with round or slightly oval nuclei containing a finely granular chromatin network. In a case of reticulum cell sarcoma, Varadi (1939) found

66% lymphocytes, 2·2% lymphoblasts and 1% reticulum cells at the first sternal puncture, and 48·6% lymphocytes, 5·5% lymphoblasts and 5·6% reticulum cells at a later one. The question arises whether this case was a combination of reticulosis and lymphatic leukæmia, as in the case described by Lösch (1932). In a case of reticulo-endotheliosis, more fully described in the section on hæmochromatosis (Case 64, p. 356), we found an increase of lymphocytes. In a case of reticulum cell sarcoma, Weil, Perlès and Fourest (1939) noted some enormous cells, 40–60 μ in diameter, in the peripheral blood and in sternal marrow. The cytoplasm was weakly basophilic and showed azurophil granules. The nucleus was large, often bizarre or sausage-shaped, and had a light and finely-granular chromatin network with nucleoli.

A special variety which interests us particularly because it may be diagnosed by marrow puncture, is Ewing's tumour, a sarcoma of the bone marrow described by Ewing in 1920 as "endothelial myeloma," or "diffuse endothelioma." It was considered to be an inflammatory reaction in children and adolescents, but Oberling and Raileanu (1932) recognized that it was in fact a proliferation of the marrow reticulum cells, corresponding to retothelsarcoma. In the sternal marrow, Carnot *et al.* (1937) observed cells with a finely-granular nucleus but with ill-defined cytoplasm. Heilmeyer (1942) found that in Ewing's tumours the whole bone marrow was infiltrated with primitive reticulum cells, some of which showed a tendency to form giant cells with 4–6 nuclei. They probably develop owing to a dissociation of nuclear and cytoplasmic division, already described by us, and an analogy is thereby provided to the leukæmias and to other types of severe marrow damage.

Summary. The reticuloses do not constitute a single uniform disease, but a group with varying ætiology and evolution. Sternal puncture may sometimes be useful for their diagnosis, but does not elucidate pathological problems concerning them. There are intermediate stages between the diffuse reticuloses without destructive growth and reticulum cell sarcoma. In view of this fact, and because other forms of reticulum proliferation are also termed reticulosis, we suggest the following classification:—

(1) Leukæmic reticulo-endotheliosis (reticular monocytic leukæmia, histioleukæmia).

(2) Acute reticulosis akin to the leukæmias (atypical monocytic and histiocytic leukæmia).

(3) Acute infectious reticulo-endotheliosis (no leukæmia, no tumours).

(4) Infectious reticulo-endotheliosis in infants (Abt Letterer-Siwe's disease), closely allied to the storage reticuloses.

(5) Chronic epithelioid-celled reticulo-endotheliosis (Boeck's sarcoidosis), and possibly also other infectious types of reticulosis

(6) Tumour-like proliferations of the reticulo-endothelial system, such as reticulum cell sarcoma and Ewing's tumour.

(7) Malignant lymphadenoma (Hodgkin's disease)

STORAGE RETICULOSES

It is now generally agreed that the disorders of storage are primarily affections of the reticulo-endothelial system, and not as Pick (1936) thought, primarily disorders of lipoid metabolism. This has been discussed by Baumann *et al.* (1936), Letterer (1938), Glanzmann and Walthard (1940) Willi (1942), and others Gaucher's disease, Niemann-Pick's disease, Hand-Schuller-Christian's disease, some of the xanthomatoses and certain cases of hæmochromatosis, belong to this group Transitional forms between reticulosis and disorders of storage have already been mentioned

Gaucher's Disease

This rare disease of lipoid storage is characterized by splenomegaly, anæmia, thrombocytopenia and leucopenia Gaucher (1882) thought that it affected the spleen exclusively. Lieb (1924) and Epstein (1937) identified the lipoid substances concerned as kerasin, a cerebroside. Schlagenhaufer (1907) brought forward evidence that the disease was familial, and affected many organs, especially the bone marrow. This, of course, suggested the possibility of its diagnosis by marrow biopsy rather than by the much more dangerous splenic puncture Barchasch and Gurin (1931) were the first to report typical findings by tibial puncture, but sternal puncture proved negative. Pittaluga and Rof (1932) also failed to find any specific changes in the sternal marrow but found them by splenic puncture. As experience grew, reports of the *findings of Gaucher cells in sternal marrow increased* Gaucher cells are large reticulum cells, 20–40μ in diameter (Schartum-Hansen (1938) reported them up to 80μ), with a relatively small, eccentric nucleus showing a coarsely trabecular chromatin structure and rarely with a few lightly staining nucleoli, the nuclei are sometimes pyknotic The cytoplasm, owing to its lipoid content, stains lightly, is faintly blue, and has a peculiar dimpled appearance, like crushed tissue paper (Klima). The wrinkles and folds are actually cavities, in which kerasin is stored Less mature cells may show a basophilic cytoplasm Klima (1938), Kienle ([illegible]), and others have observed cells with two or three nuclei, which must be taken as indicating abnormalities of the mitotic processes. Owing to the foamy cytoplasm, Rohr (1940) coined the term "foam cells." Marrow puncture may be technically difficult in these cases because of the hyperplasia of the reticulum, and usually only a very little marrow juice is obtained Gaucher cells are sometimes found in

cutaneous localization, such as xanthoma disseminatum or tuberosum multiplex, often referred to as "lipoid gout" because it occurs near the joints, and xanthosis and xanthelasma. *Some forms show* predominantly visceral localization with hepatic, pulmonary or cardiovascular *lesions. Marrow biopsies have only rarely* been recorded in these affections. Michaud and Sorba (1943) reported a case *of cholesterolosis of the skin, probably* also affecting the liver and the endocrine glands, with a cholesterolæmia of 2,000 mg. %. *The sternal* marrow showed a increase of reticulum cells up to 22%, and 10% were xanthomatous cells. In a patient with generalized xanthomatosis and a cholesterolæmia of 1,100 mg. %, Thaddea (1943) found an increase of fat storage. The cells described correspond to the so-called foam cells.

Summary. Sternal puncture can frequently be a useful aid *to the diagnosis of the reticuloses* with *disturbance of storage.* Localization differs in the various diseases. Gaucher's disease appears to affect the bone marrow much earlier and more frequently. The differentiation of the various reticuloses with constitutional disturbances is not possible by the method of sternal puncture, but *once the presence of some reticulosis is proved in any* given case, it is possible to make a diagnosis on the basis of clinical *symptoms. In Hand-Schüller-Christian's disease, puncture* at the site of the bony change is recommended.

Hæmochromatosis

Hæmochromatosis is a variety of reticulo-endotheliosis in which blood *pigment is stored, instead of lipoids,* as in other forms. While on the basis of recent investigations, the lipoidoses are *recognized as primary reticuloses,* the majority of cases of hæmochromatosis are primarily due to an increase of hæmolysis, and *the reticulum hyperplasia is merely secondary. There are,* however, cases in which reticulosis is the primary process, or at any rate dominates the pathological *picture to a remarkable* degree

Case 64. T W., a man of 45 years, had suffered from sciatic pain on and off for 20 years. Eighteen months before admission he had had an accident involving the knees During the last 2 months he complained of lassitude, a feeling of fulness and of *malaise, and there* was a yellow tinge of the skin. On admission, he was a muscular, well-*nourished man,* with a bronzed skin. Heart and lungs, n.a.d Blood pressure 120/70 mm. Hg. Electrocardiogram normal Liver enlarged to the umbilicus Spleen enlarged to 4 in. below the costal margin. Temperature up to 104° F. Urine: urobilin +, urobilinogen +, *diazo-*reaction negative, few polymorphs in the sediment

Blood. *R B C* 3 58 *millions,* Hb. 77% = 12 3 g %; W B C 9,200, [illegible] lympho-
eosinophils 1·5%, stab forms 2%, [illegible]
cytes 47%, *monocytes* 1·5%. Ser[illegible]
reaction negative. Sedimentation [illegible]
2 hr)

From the blood picture an atypical mainly hepatolienal type of lymphatic leukæmia seemed the most likely diagnosis, especially as there were a number of primitive forms among the lymphocytes with only one nucleolus In one part of the smear we found a small group of three reticulum cells.

STERNAL MARROW. Proerythroblasts 0 6, early basophilic normoblasts 1·3, normoblasts 17 per 100 white cells; myeloblasts 1·3%, promyelocytes 3%, semimature myelocytes 2 3%, mature myelocytes 10%, metamyelocytes 11·3%, stab forms 17 6%, segmented polymorphs 24 3%, basophil myelocytes 0 6%, basophils 0 6%, eosinophil myelocytes 0 6%, eosinophil metamyelocytes 1·3%, eosinophils 1%, lymphoblasts 1%, lymphocytes 22 6%, monocytes 0 6%, megakaryocytes 0 3%, plasmoblasts 0 3%, proplasmocytes 0 3%, plasma cells 1%, ...m cells 1%, phagocytic reticulum cells 1·3%, endothe- ...ather poorly cellular. From the myelo- ...ost probable one, owing

I however, helped to
l inophils 0 5%, stab

to . The further de... leukæmia ..., lymphocytes 23%, monocytes exclude lymphatic leukæmia segmented polymorphs 65%, hepatomegaly (4,700 g.) and forms 8 5%, plasma cells 1%. At autopsy enlargement of the abdominal 2%, splenomegaly (680 g) were found and Histological examination lymph glands, some of which were softened with pigment cirrhosis as well as showed generalized hæmochromatosis reticulo-endothelial system with deposits generalized proliferation of the organs, including the bone marrow. of hæmosiderin in nearly all the

The diagnosis in this case could not be made by sternal puncture, because the reticulum cells were not increased A case of reticulum cell sarcoma described by Varadi (1939) is worth mentioning There was considerable lymphocytosis in the marrow (66% lymphocytes and 2 2% lymphoblasts and 48 6% lymphocytes and 5 6% lymphoblasts, respectively, in the two sternal punctures). In our case the presence of jaundice and the slightly raised serum bilirubin level favoured hæmolysis as the ætiological factor, but the reticulum proliferation was the chief pathological feature Markoff (1938) reported a case of hæmosiderosis in which the Prussian-Blue reaction was positive in the marrow

HODGKIN'S DISEASE (LYMPHADENOMA)

The ætiology of this disease is still uncertain Before Sternberg's (1936) description all sorts of cases were thought to be " pseudoleukæmia," or Hodgkin's disease In Hodgkin's (1832) own series, only 4 cases definitely belong to the disease now named after him Paltauf (1912) and Sternberg (1936) believed that some relationship existed between lymphadenoma and tuberculosis, but this view has since been discarded for lack of corroborating evidence (Leitner, 1935) Hueck (1936), van Rooyen (1937), Medlar *et al* (1937) and others suggested its neoplastic nature, recently revived by Eskola (1940), who stated that the measurements of the nuclei and the nucleoli favoured this theory Following Gordon's (1936) experi-

ments, which resulted in the development of the test named after him, lymphadenoma was regarded by many as a virus disease. The encephalitic phenomenon in rabbits after the intracerebral injections of an emulsion of lymph glands was confirmed by Friedemann and Elkeles (1933), Hoeden and Hulst (1934), van Rooyen (1937), Uhlenhuth and Wurm (1940) Though the value of the test is established, the reaction is, of course, not specific for lymphadenoma. Positive results may be attributed to the eosinophil cells present in the gland (Friedemann and Elkeles, Uhlenhuth and Wurm). Morphological observations and the usually progressive, almost malignant progress of the disease, favour the theory of its neoplastic nature. Histological observations, on the other hand, would indicate some relationship to the reticuloses. The course of the disease is usually chronic, with remitting pyrexia (Pel-Ebstein phenomenon), but fever may be entirely absent. Acute forms have been described: Fontein, Smeets and Straub (1941) recorded 2 cases, which survived only 10 and 24 days respectively. Histological examination showed an early reticulo-endotheliosis. The clinical symptoms are: loss of appetite, loss of weight, bouts of sweating, pruritus, pulmonary manifestations, and enlargement of the lymph glands, which may reach considerable size In one of our cases, enlargement of the supraclavicular and cervical glands was so marked that the patient became severely disfigured and his features were almost unrecognizable.

The blood picture is characterized by leucocytosis with relative lymphopenia and eosinophilia. There is often monocytosis. Thaddea (1943) believes that a really typical blood picture is seen but rarely Gebauer (1939) considers that severe anæmia is infrequent even in advanced cases In our experience there is no characteristic blood picture in lymphadenoma, but the most constant feature is lymphopenia In these circumstances some definite assistance from sternal puncture in establishing a diagnosis would have been welcome Specific lymphadenoma tissue might be expected, because foci of the disease occur in the bone marrow in about half the cases (Mittelbach-Schmidt and Stolz, 1937; Symmers and Hutcheson, 1939) Dresser (1926) believes that the sternum is affected relatively frequently. Askanazy (1927) describes two forms: circumscribed nodules and specific jelly-like atrophy. Marchal *et al* (1941), in a series of 42 cases found bony involvement six times and 3 of these in the sternum They attribute anæmia to an increased destruction of erythrocytes in the spleen and to crowding out of the marrow by lymphadenoma tissue. Piechl (1941) reported a case localized exclusively in the bone marrow Camerini (1941) observed a case of primary lymphadenoma situated in the 4th dorsal vertebra, and in the 9th to 11th ribs In spite of these findings, specific lymphadenomatous tissue is seen only rarely in the marrow Weiner

and Kaznelson (1926), Nordenson (1935), Markoff (1936), Barasciutti (1937), Jagić and Klima (1937), Schulten (1937), Gasbarrini (1939), Henning and Keilhack (1939), Velasco-Montes (1939), Fieschi (1940), Leitner (1940), Thaddea (1943) and others, all recorded negative results Dameshek (1935) observed an increase of histiocytes up to 24%, but Klima (1938) believed that these cells were of a myeloid nature Rohr and Hegglin (1936), in one case, found large cells with large nucleoli, which they thought were *Sternberg's* giant cells, but this was not at all certain. Fleischhacker and Klima (1937) described a type of cell first observed in material from lymph gland puncture, characterized by a large amount of greenish-blue, lumpy and vacuolated cytoplasm. The nucleus was large and loosely knit and showed one or more nucleoli. This cell, designated a "lymphadenoma cell," may become enlarged, its nucleus then becomes lobed and corresponds to the description of Sternberg cells Signs of degeneration increase with increasing size Because the development is not equal in all the cells, a pleomorphic picture results *Lymphadenoma* cells predominate in the glands, while myeloid and reticulum cells are decreased in numbers The number of lymphadenoma cells in sternal marrow obtained by biopsy is small, but according to Klima the immaturity of the lymphoblasts in the marrow is a remarkable feature. In several cases of Hodgkin's disease we have seen the cells described and pictured by Klima (Fig 181) We are, however, not convinced that these cells are in any way specific. They resemble atypical plasma cells fairly closely Weber and Huber (1939) observed large cells in the marrow with round nuclei, about 7μ in diameter, such as are found in reticulosis Kienle (1943) found definite lymphadenoma cells in only 5 of his 25 cases



Fig. 181 Lymphadenoma cell (of Klima) in sternal marrow in Hodgkin's disease ("Atypical plasma cell") (×1,000)

Kienle gives instructive pictures of the cells, which are characterized by several nuclear fragments subdivided like a bundle of sausages, staining a bluish-red colour in the ordinary Giemsa preparation and containing large, purple nucleoli Their cytoplasm is bluish, scanty and often vacuolated, and does not contain granules. He also noted large mononuclear cells with a rather coarser nucleus with small nucleoli, and a broad rim of cytoplasm Pathognomonic findings by marrow puncture are certainly uncommon Puncture of the lymph gland is of greater diagnostic value (Stahel 1939, Velasco-Montes, 1939, Leitner, 1940)

Other marrow findings are as follows Nordenson, in one case, noted a shift to the left of the myeloid cells and also toxic granulation. Klima found increased numbers of cells, and Dameshek an increase of myeloblasts Landolt (1944) observed an eosinophilic leukæmoid picture in lymphadenoma with 90% eosinophils in a total number of

122,000 leucocytes, and in this case the marrow also showed eosinophilia. In the case described by Major and Leger (1939), eosinophilia was even more pronounced (99% of a total leucocyte count of 169,000). A myelocytic reaction occurs according to Klima in advanced cases, and according to Markoff in pyrexial cases, but the reticulum cells remain unchanged. Eosinophilia is is more often seen when the skin is involved. In our experience it is untreated cases which have increased cell counts. Late cases show a more or less marked shift to the left, an increase of plasma cells and eosinophilia. Mallarmé (1937) reported an increase of reticulum cells and plasma cells. We have seen eosinophilia, and it has also been observed by Barasciutti (1937) and de Weerdt (1939), even in cases without eosinophilia in the peripheral blood. Révol (1938) collected a series of 3 cases and found increases of myelocytes and frequently of normoblasts, but the *myeloblasts were diminished. Fieschi and Reittanni (1938)* examined 8 cases, 4 of which were not treated by radiotherapy. They found the normoblasts rather than the granular series increased, with a lag type of maturation curve and occasional increases of lymphocytes and plasma cells, and often also of histioid cells. The eosinophils were either normal or increased. In a case treated by X-rays they noted numerous hæmocytoblasts with many mitoses. Rütte (1944) observed a case, which initially gave the histological picture of an extramedullary plasmocytoma, with 14% paramyeloblasts in the sternal marrow.

Case 65. M M, a girl of 19 years, became ill in July, 1935, with a productive cough and pain in the side Pulmonary tuberculosis was [illegible]

at that time In 1938 she [illegible]

[illegible] Because of this treatment-resistant [illegible] University Dermatological Clinic in [illegible] was excised and Hodgkin's disease diagnosed

BLOOD (July, 1938). R B C 5 08 millions, Hb. 100% = 16 g %, W.B C. 7,900, eosinophils 7%, segmented polymorphs 65 5%, stab [illegible] monocytes 6%, sedimentation rate [illegible] nd 3 hr)

[illegible] Proerythroblasts 0 75, early baso- [illegible] 15·25 per 100 white cells, myelo- [illegible] semimature myelocytes 3 25%, [illegible] 10 5% stab forms 24 25%, [illegible] eosinophil [illegible] monocytes [illegible] asts 0 5%. Very occa-

myeloblasts 1 5%, promyelocytes 9%, semimature myelocytes 7 25%, mature myel b forms 15 5%, segmented p 3%, eosinophil metamyelocy 1 75%, monocytes 1·5%, %, plasmoblasts



Fig 182 Plasma cell proliferation in sternal marrow, in lymphadenoma (Hodgkin's disease) (×1,400)

In this case, open active pulmonary tuberculosis co-existed with Hodgkin's disease The Mantoux reaction became negative during the development of lymphadenoma The tuberculous process subsided under the influence of artificial pneumothorax treatment and did not become active again as the lymphadenoma progressed. While the radiographic picture of the mediastinum

slight eosinophilia and lymphopenia, but subsequently leucocytosis developed with a shift to the left and marked lymphopenia (5 5%). Slight anæmia also became noticeable The first sternal puncture showed hypoplasia of erythropoiesis and a slight metamyelocytic shift to the left, but in the second puncture the myelocytic shift to the left became definite, eosinophilia and plasma cell proliferation developed and a few lymphogranuloma cells of Klima's type were seen (Figs. 181 and 182).

It is not certain whether the presence of these cells is a specific sign of lymphadenoma, or whether they are, in fact, atypical plasma cells. In material obtained by puncture of the lymph glands from the same case we found similar lymphogranuloma cells, and lymphoblast-like and monocytoid cells, but no typical Sternberg giant cells. In other cases, we usually found Sternberg cells by puncture of the lymph glands. The diagnosis in this case was established by gland biopsy.

Summary. Sternal puncture shows the specific finding of Sternberg cells in lymphadenoma only rarely. In our 9 cases, we saw a more or less definite shift to the left (which in case 65 became more marked during the progress of the disease), eosinophilia and plasma cell proliferation The latter is usually more pronounced in late cases, which also develop slight anæmia with hypoplasia of erythropoiesis in the marrow.

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CHAPTER XIII

TUMOURS

TUMOURS of the marrow stroma, *e.g.*, multiple myelomatosis, the reticuloses and reticulum cell sarcoma, and also Ewing's sarcoma, have been discussed in previous chapters. In the case of secondary malignant deposits in the marrow, sternal puncture can establish a diagnosis only when metastases are already in the sternum, *i.e.*, in late cases. Sternal puncture may, however, be of some value, because a clinical diagnosis cannot always determine the presence of small metastases. This is illustrated by Case 20 (p. 172), a case of leucoerythroblastic anæmia in carcinomatosis of the bone marrow, and by Case 66 (p. 367). Pässler (1931) demonstrated that carcinomatous deposits are more commonly localized in the manubrium of the sternum. When a growth is suspected, the manubrium, rather than the body of the sternum, should be punctured. In all our cases we found puncture easy, but in osteoplastic types of carcinomata it may be very difficult. In such cases only very little material is obtained, and in the scanty, rather poorly cellular marrow, groups of tumour cells may be found (Leitner, 1945) Grunke (1938) found that the tumour tissue may often be found in the needle even if it does not reach the barrel of the syringe Henning (1935), Schulten (1938) and Leitner (1945) recommend that the site of clinical signs of growth should be punctured The vertebræ, the ileum, or the ribs may be examined when they are suspected as sites of metastases. Naegeli (1931), Rohr and Hegglin (1936), Vogel, Erf and Rosenthal (1937), Klima (1938), Markoff (1938), Schulten (1938), Nordenson (1938), Vischer (1938), de Weerdt (1939), Gormsen (1940, 1942), Kreyberg and Poppe (1940), Tischendorf and Frank (1940), Stöger (1941), Francke (1942), Mallet and Gasne (1942), Kienle (1943), Thaddea (1943), Leitner (1945), and others have all found pieces of growth by sternal puncture. The number of negative findings reported is much smaller (Dameshek, 1935; Jagić and Klima, 1937, Monasterio, 1939)

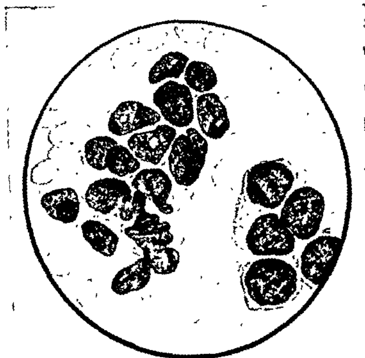
The frequency of positive marrow findings varies according to the selection of the patients for any particular series. In a series of 74 patients, 12 of whom were suspected of having metastases in the marrow, Rohr and Hegglin (1936) found groups of carcinoma cells by sternal puncture on 10 occasions Kreyberg and Poppe (1940), in 100 cancer cases, demonstrated the presence of carcinoma cells in 8 patients by sternal puncture Gormsen (1940) collected a series of 131 growths (111 carcinoma and 21 sarcoma). In 8 cases tumour cells were seen in sternal marrow, 7 of these came from

39 patients with carcinoma of the breast. Subsequently he (1942) reported his experience of 263 cases of malignant disease In 56 cases (18%) he found tumour cells in the sternal marrow. In 110 cases of malignant disease, Stöger (1941) found tumour cells in the marrow of 8. He also observed plasma cell hyperplasia of 1·1%–16% and a myeloid shift to the left. Francke (1942) succeeded in demonstrating tumour cells in the sternal marrow in 50% of cases with marrow metastases. He believes that the diffuse deposition of single tumour cells is very rare, but that groups of tumour cells can often be found. He obtained 20 positive findings in 134 cases. Mallet and Gasne (1942) reported a positive finding in 15 out of 35 cases. In our 39 cases (Leitner, 1945), we obtained only 3 positive results. Selberg (1943) reviewed 115 autopsies and found only 13 cases with bony metastases. The sternum was affected in half of these. The caudal and dorsal parts of the sternum were the most usual sites.

Several authors have reported on the morphology of tumour cells obtained by sternal marrow biopsy. Quensel (1928) and Zadek (1933) reported that carcinoma cells have large nucleoli in proportion to the nuclei (0·3μ–0·44μ instead of 0·1μ–0·2μ). The cells are usually large, about 30μ. The proportion of cytoplasm to nuclear volume varies considerably, but the nucleus is usually the larger (Klima, Leitner). The chromatin structure is loosely knit, resembling a coarse, knitted net of threads, set against a background of colourless ground substance. The cytoplasm is dull blue and often vacuolated. The outlines of the cytoplasm are often indefinite, and often hardly recognizable in cell groups The cytoplasm is usually homogeneous, but it may show, as illustrated by Fig. 183, an uneven granulation which is rarely plentiful, but often rather coarse Mitotic figures are frequent Abnormal mitoses, resulting in binuclear cells (nuclear division while the cytoplasm does not divide) and manifestations of degenerative changes, are frequent Apart from the larger forms, there are also smaller malignant cells In these the pleomorphism of the nuclei is less apparent, and nucleoli may be absent (Rohr and Hegglin, Markoff, Schulten) We found definite nucleoli also in the smaller cells. The nucleus is usually finely marked, sometimes mottled Mitoses are less common Rohr and Hegglin (1936) found the smaller forms commonly in bronchial carcinomata, but Kienle (1943) and Klima (1938) state that the larger forms predominate in bronchial carcinoma. The distinction of large and small-celled forms is not always possible, and actually is not important as long as the malignant cells are recognized as such Diagnosis is often only possible by the use of histological preparations and we recommend that part of the material obtained by sternal puncture should always be embedded in paraffin, Kreyberg and Poppe (1940) express the same opinion When puncture material is scanty,

PLATE VI

Cluster of Carcinoma cells in the Sternal Marrow



The individual cells show much variation in size and shape. Some nuclei contain nucleoli. The cytoplasm is ill defined. Groups of cells such as these are easily recognized as elements foreign to normal bone marrow.

smears may show isolated groups of tumour cells, while in sections they may be difficult to find because they are so small. Marrow smears provide a much better view of the finer morphology, but the diagnosis is more certain by histological methods. Francke (1942) considers the pleomorphism of cell size and nuclear configuration, as well as the rapid absorption of the stain in supravital methods (Sudan-Cadmium, Cadmium-methylene blue, Quensel's method), favour the diagnosis of neoplastic cells. Differential diagnosis between carcinoma and sarcoma can be decided only by histological sections. Tischendorf and Frank (1940) state that spindle cell sarcomata may be diagnosed on smears with some degree of accuracy. The site of the primary growth can never be diagnosed by smear preparations (Rohr and Hegglin, Leitner). Kienle distinguishes six forms —

(1) Large tumour cells with round nucleus, fine chromatin structure and a broad rim of light blue cytoplasm.

(2) Smaller cells with round nucleus, with irregular finely-meshed nuclear structure and light blue cytoplasm.

(3) Small round cells with coarse nuclear structure, nucleoli and a well-preserved, basophilic perinuclear halo of cytoplasm.

(4) Plasma cells with very fine nuclear structure, with large nucleoli. Their nuclei are oval or irregular in shape.

(5) Large tumour cells with round or oval nucleus, of a coarser structure and with numerous nucleoli.

(6) Giant cell sarcoma.

We feel that it is much more important to find cell groups rather than to classify individual cells. Clusters of cells permit a reasonably reliable diagnosis, but individual cells are not always easily distinguished from primitive marrow cells, especially marrow reticulum cells (Nordenson, Tischendorf and Frank, Thaddea, Leitner). The following case is an example of the large-celled forms —

Case 66. C A, a watchmaker, aged 63 years, had had pain in the chest since a motor accident. During the last 2 months the pain increased in severity, he lost his appetite and much weight. When examined, he was wasted. The heart was slightly enlarged to the left. A radiograph of the chest showed an irregular ring-like shadow in the left mid zone. Hard palpable glands were present in the supraclavicular fossa. The thyroid was slightly enlarged and hard.

BLOOD R B C 4 37 millions, Hb 88% = 14 2 g %, W B C 6,920, basophils 2%, segmented polymorphs 78%, lymphocytes 11%, monocytes 7%, sedimentation rate (Westergren) 27–71 mm (1 and 2 hr) Blood cholesterol 156 mg %, serum protein 8 3 g %.

STERNAL MARROW Proerythroblasts 0 5, early basophilic normoblasts 0 25, normoblasts 9 per 100 white cells, myeloblasts 1%, promyelocytes 2 75%, semimature myelocytes 2 75%, mature myelocytes 7%, metamyelocytes 17 25%, stab forms 17 5%, segmented polymorphs 24%, basophils 0 75%, eosinophil myelocytes 2%, eosinophil metamyelocytes 1 75%, eosinophils 2%, lymphoblasts 3%, lymphocytes 10 5%,

monocytes 1·5%, megakaryocytes 0·25%, endothelial cells 0·75%, lymphoid and phagocytic reticulum cells 5·5%. A group of tumour cells was also seen (Fig. 183). The patient did not survive long. Autopsy revealed carcinoma of the bronchus.

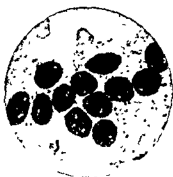


FIG. 183 | Group of carcinoma cells in sternal marrow in a case of bronchial carcinoma (×500)

In this case the sternal puncture showed hypoplasia of erythropoiesis, a myelocytic shift to the left, an increase of the reticulum cells and a group of tumour cells. The cells had a vesicular, lightly staining nucleus with fine, loose chromatin structure on a light ground substance. The large nucleoli were light blue and contrasted well against the nuclear chromatin. The large-celled form was also apparent in a case of carcinomatosis with metastases in the marrow, resulting in a leuco-erythroblastic reaction (Leitner, 1945). As illustrated by Figs. 184 and 185, the sternum, ribs and the vertebræ were punctured and showed similar cell groups.

In the following case of the small-celled form, sternal puncture proved negative, but puncture at the site of election, in this case the greater trochanter, showed extensive groups of tumour cells. The patient had had sciatica pain for some time.



FIG. 184 Bone marrow puncture Group of carcinoma cells from a case of carcinoma of the stomach (×750)

... 1936 with ... ecame ... eight, ... loctor sweated a good deal and had to take to his bed. ... diagnosed rheumatism and suggested spa treatment. This was unsuccessful. The patient consulted a neurologist, who ordered ultra-violet light therapy. In December, 1937, the right side of the face became

paralysed. In the spring of 1938 he had further spa treatment, again without improvement, in the autumn the paralysis became more pronounced. When examined, he was wasted and pale. There was right-sided facial and hypoglossal paralysis. The tendon and periosteal reflexes were increased, but there was no evidence of any pyramidal tract lesion In the mitral area a systolic murmur was heard. An X-ray of the chest showed a definite oval shadow in the left mediastinum. B.P. 155/80 mm Hg



Fig 185 Groups of carcinoma cells in sternal marrow in a case of carcinoma of the stomach. (×750)

Blood R.B.C 3·9 millions, Hb 75% = 12 g.%, C.I. 0·95; W.B.C. 6,660; eosinophils 0·5%, stab forms 3%, segmented polymorphs 86%, lymphocytes 8·5%, monocytes 2% Sedimentation rate (Westergren) 42–71 mm. (1 and 2 hr)

Sternal Marrow (December 9th, 1938). Normoblasts 12 per 100

Because the patient continued to complain of pain in the right thigh, the right greater trochanter was punctured, where it was tender to pressure. Part of the material obtained was examined histologically.

Smears from Femoral Marrow (January 13th, 1939) Early basophilic normoblasts 0·6, normoblasts 26 per 100 white cells, myeloblasts

This was a case of bronchial carcinoma confirmed by autopsy, with metastases in the greater trochanter and in the skull bones, and in which the peripheral blood showed only slight anæmia and some degree of lymphopenia. The sternal marrow revealed hypoplasia of erythropoiesis and a slight increase of reticulum cells, but there was no definite shift to the left of the granulocytes. Puncture of the trochanter, performed 5 weeks later, showed a normal number of normoblasts, a considerable myelocytic shift to the left, an increase of reticulum cells and megakaryocytes, and also many groups of tumour cells (Figs. 186, 187).

The cells were of the small type. Their nuclei were frequently oval and many showed definite nucleoli. The chromatin structure was fine The cytoplasm was weakly basophilic and often indistinct.

The increase of *megakaryocytes in the* marrow was remarkable, all the more so as the platelet count was 768,000 per cmm. This case had been *reported elsewhere as an example of secondary* thrombocythæmia. It was especially interesting because thrombocytopenia with a *hæmorrhagic diathesis is a very much more usual* finding in malignant disease (Kurpjuweit, 1903; Dunner, 1921; Blum, 1928; Schildknecht, 1939; *Canali, 1942; Kienle, 1943;* Leitner, 1945). The marrow giant cells are mostly normal (Blum), decreased or degenerate (Kienle), or even absent *(Schildknecht).* We have not found another case of thrombocythæmia in malignant



FIGS. 186 and 187 Groups of carcinoma cells in trochanteric marrow from a case of bronchial carcinoma, in which sternal puncture did not show such cells (×500)

disease reported in the literature. Other marrow and blood findings need further discussion. The question arises whether the diagnosis of carcinoma may be arrived at without the actual demonstration of tumour cells. Markoff (1938) believes that the change in the structure of the bone marrow does not depend so much on the site and the type of the primary growth as on the state of the marrow. It may result in marrow hyperplasia (osteoporosis) or marrow hypoplasia (osteosclerosis). He has *observed an aplastic reaction in a poorly cellular marrow in one case,* but Hirschfeld (1920), Sonnenfeld (1929), Naegeli (1931) and *Domarus (1937) have usually found hyperplastic reactions* Weinberg (1921) states that carcinoma of the stomach is accompanied by leucocytosis and the release of myelocytes into the circulation in 38·5% of the cases.

[illegible] described by Diaballa and Entz (1913) (W. [illegible] by Domarus (1937, W B C. 96,[illegible] 70,000; myelocytes 18%); by Muller (1938; W B C. 64,000; myelocytes 6%, and another case of an old woman with a white cell count of 50,000) In a case described by Forconi and *Carere-Comes (1940) the clinical* picture of myeloblastic leukæmia was simulated. Plenge (1937) states that *the blood and marrow pictures* are almost normal, unless some infection has supervened When this occurs, mylo-

blasts and myelocytes increase. This view tallies with Arneth's (1942) opinion, attributing leukæmoid reactions to pus formation within the growth. Hadorn's case (1944) of a carcinoma of the lung is interesting. It showed 75,000 white cells, 43% being eosinophils, but the marrow showed no sign of leukæmia. Mallarmé (1937) examined 3 patients by marrow biopsy. Only once was a myelocytic marrow seen, but in a case with leucocytosis in the peripheral blood there were also many segmented polymorphs. Nordenson (1935), in 7 out of 10 cases of carcinoma of the stomach, observed leucocytosis in the blood, and in the marrow a myeloid shift to the left with increases of myelocytes, promyelocytes and myeloblasts. The numbers of erythroblasts were either reduced or increased. In our 39 cases, a myelocytic shift to the left was seen in 24. In none of the cases was there a definite increase of myeloblasts, and in only five an increase of promyelocytes. Eosinophilia in the blood and marrow was seen in 4 cases. In 142 cases of malignant disease Annoni (1941) recorded eosinophilia on 15 occasions. This should be regarded as an allergic reaction to protein bodies, set free by necrotizing processes in the tumour. Kurpjuweit (1903) and Diaballa and Entz (1913) hold that a myeloid shift to the left indicates skeletal metastases, and we believe that it should make one suspect metastases in the hæmopoietic organs, *i.e.*, in bone marrow, spleen and liver Naegeli (1931) did not consider that the myeloid shift to the left is a mechanical reaction, that is, due to crowding out of hæmopoietic tissue, but a biological one which occurs mainly in younger patients This view is contradicted by Muller's (1938) case of an old woman of ninety years Myeloid reactions have also been recorded by Kugelmeier (1935), Andreu-Urra and Regli (1937) and Arneth (1942) They are not very frequent, and in 110 cases of carcinoma Stöger (1941) found only one. Kast (1941) reported a case of carcinoma of the stomach with metastases in the liver, which showed a lymphatic leukæmoid reaction. Like Koch (1931), we found lymphopenia in cases with bony metastases Weinberg (1921) states that lymphopenia indicates carcinoma when there is a suspicion of carcinoma of the stomach, but that cases with achlorhydria and without carcinoma show leucopenia with relative lymphocytosis Sonnenfeld (1929) and Zadek (1933) noted leucocytosis with neutrophilia, a shift to the left and relative lymphopenia in carcinomata which destroy bone, but leucopenia with relative lymphocytosis in carcinomata which cause formation of bone

Normoblastic crises have also been observed in cases of malignant disease Weber (1940) reported erythroblastæmia in a case of carcinoma of the stomach with skeletal metastases, and believed that this was of diagnostic significance. Loeper, Mallarmé and Brault (1939), in a case of miliary spread of carcinomatosis of the lungs and metastases in the bone marrow, observed anæmia with 60% normoblasts in blood and marrow. In very rare cases

leuco-erythroblastic reactions have been seen, as by Mach a Klages (1930), Moeschlin (1940) and Leitner (1945). Leukæm or leuco-erythroblastic reactions in the peripheral blood are d *not only to the leakage of immature cells through the bone marro blood barrier, but also to extramedullary hæmopoiesis, clea* demonstrated by Mach and Klages (1930), Buchem and Hendriks *(1939) and Taylor and Smith (1941). In Taylor and Smith's cas* just as in ours, the marrow was aplastic or hypoplastic, but t *enlarged spleen showed tremendous myeloid metaplasia. Li* Rohr *and Moeschlin, we regard this as a compensatory reaction.*

Illustrations of sarcoma giant cells have been published l Fleischhacker and Klima (1936), Klima (1938), Rohr (1940), ar Kienle (1943). Kienle's photomicrographs clearly illustrate tl marked nuclear pleomorphism of the metastatic growth. Nucleo which are unusually large for tumours, marked differences of c and nuclear size, *varying from dwarf to giant forms,* uneve staining of the cytoplasm and signs of degeneration are als recognizable. The development of multinuclear giant cells ca be traced by the mitotic figures and followed through nuclea division, while the *cytoplasm remains undivided. Battle an* Stasney (1941), Dicker and Dubois-Ferrière *(1942) have note pigment containing cells in cases of malignant melanoma.* Markol (1942) found reticulum cells containing hæmosiderin in a case o *osteitis fibrosa cystica. Albertini and Willi (1938), de Weerdt (1939* and Hooft and Campernolle (1942) have diagnosed neuroblastoma *by sternal puncture. In the last-mentioned case the demonstration* of the specific cells, described by Kato and Wachter (1938) in the *sternal marrow, was possible even before clinical or radiological* manifestations appeared. In a case of leukosarcomatosis, Quattrin *(1939) did not see any definite changes. In lymphosarcoma,* Dameshek (1935) reported a suppression of the normal marrow by lymphoblasts and lymphocytes. We have seen 3 *cases of* lymphosarcoma in 2 of which the marrow was normal. In the third case there was a slight *increase of lymphoid reticulum cells, and a slight lymphatic hyperplasia without, however, any appreciable suppression* of hæmopoietic marrow.

Case 68. H. E, a newspaper seller of 60 years, had had asthma, weakness in the left hand with the fingers becoming thinner during the last year. The patient's mother died of carcinoma of the stomach During the last few weeks she had lost about 8 kg in weight, *was short of*
[illegible] Her ankles began to swell and she com-
[illegible] *was enlarged The*
[illegible] damage, *e g*, low
voltage curve, a rather [illegible] ding of R-wave in
leads I and II and in the S-wave in lead [illegible] and IV. *Dulness and*
rhonchi were present at the right base. [illegible] picture of the chest
showed irregular shadows [illegible] the left [illegible] nsive loss of trans-
lucency in the right [illegible] mid zo[illegible] hy of the gastro-

intestinal tract showed that the transverse colon was pushed upwards. In the pancreatic region there was a mass the size of a child's head. [illegible] uterus, [illegible] juice, [illegible] ytosis [illegible] 0·5%, stab forms 4%, segmented polymorphs 78%, lymphocytes 6%, monocytes 10%, plasma cells 0·5%. Sedimentation rate (Westergren) 51–87 mm. (1 and 2 hr.) Blood urea 22 mg.%, later on 32 mg.%; Bilirubin, direct and indirect, negative. Blood cholesterol 91 mg.%; Takata-Ara reaction negative.

STERNAL MARROW. Proerythroblasts 0·5, early basophilic normoblasts 2·5, normoblasts 31·25 per 100 white cells, myeloblasts 2·75%, [illegible] 0·5%. The diagnosis at autopsy was: lymphosarcoma with metastases in the vertebræ.

Dameshek, who believes these cases show great similarity to lymphatic leukæmia, speaks of lymphatic transformation of the marrow. This case 68, on the other hand, does not confirm Dameshek's view. Apitz (1940) stated that the differential diagnosis between lymphatic leukæmia and lymphosarcoma cannot be made by the histological examination of a piece of marrow obtained by biopsy, but that a blood picture and sternal puncture are absolute necessities. Thus we see that clinico-pathological investigations and histological examination are complementary methods of approach.

Summary. Sternal puncture enables us to make the diagnosis of malignant growths in about 10% of the cases, but it is not an early diagnosis, as such cases must have skeletal metastases. However, marrow biopsy does often contribute towards a definite diagnosis before other symptoms suggestive of metastatic growth appear (cases 20 and 66, pp. 172 and 367). Puncture at the site of election, *i.e.*, where skeletal metastases are suspected clinically, increases the percentage of positive findings (see case 67). The tumour cells are foreign to the marrow and mostly recognizable, but groups of cells are necessary in order to make the diagnosis. Histological examination of a part of the material obtained by marrow puncture increases the reliability of diagnosis. In the presence of skeletal metastases, leukæmoid, erythroblastic and, more rarely, leuco-erythroblastic reactions are seen. Sometimes the marrow is transformed correspondingly, and sometimes there is a hypoplastic marrow reaction with compensatory extramedullary hæmopoiesis. In the vicinity of metastases in the marrow, disturbances of maturation may occur owing to the toxic effect

of the tumour. The red corpuscles may show punctate basophilia, as reported by Markoff. We have found this especially in the erythroblastic reactions, and in our experience it is not always confined to the areas immediately surrounding the malignant deposit. We have also observed giant stab forms, giant myelocytes and giant promyelocytes and even changes in the plasma cells. Kienle has observed the development of supplementary nuclei as well as the formation of giant myeloid cells.

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CHAPTER XIV

STERNAL MARROW IN LIVER DISEASE

THE importance *in hæmopoiesis of the liver as the organ of* storage of the anti-pernicious-anæmia factor has been discussed in Chapter VIII, *and its importance in leucopoiesis in Chapter IX.* Like Schulten and Malamos (1932), Fellinger and Klima (1934), and Schalm (1938), we have found *cases with macrocytosis in liver* disease. Schalm (1938) believes that any considerable damage to the liver leads to an increase in the size of erythrocytes; in catarrhal jaundice by about 1μ and in obstructive jaundice by about $0{\cdot}7\mu$. This, he claims, is of importance in the differential diagnosis. In cases of cirrhosis of the liver, Naegeli (1931) found a fatty marrow with poor maturation of the myeloid cells and vacuolation of the more mature forms. Schulten (1937), Skouge (1937), Markoff (1938) and Rohr (1940) report increase of pigment-storing and lymphoid reticulum cells. Schulten, however, did not think that this increase was of diagnostic importance; nor does he consider anisocytosis of red and white cells of diagnostic value. Markoff believes that macrocytosis may occur in severe *hæmosiderosis and in other states associated with choking of the* reticulo-endothelial system. Klima (1938) observed hæmoglobinized *normoblasts with much cytoplasm in sternal marrow.* He believed that they were the reason for the macrocytosis. Tischendorf (1938) investigated 38 cases of liver disease. He found that the severity of the liver disease in the various disorders was reflected accurately by increased erythropoiesis, an increase of the reticular plasma cells and by pigment phagocytosis. The reactive hyperplasia of the reticulum is attributed to a serous exudate in the tissue. The similarity between plasma cells and early normoblasts is pointed out and the possibility suggested that plasma cells may be precursors of normoblasts under the influence of derivatives of hæmoglobin set free by the destruction of phagocytes, but we cannot accept these views. *In inflammatory diseases of the liver,* especially when complicated by ascites, there is often a myelocytic reaction, *which at times may severely suppress the erythroblastic* reaction. The myeloid cells may show toxic granulation and vacuolation.

In cases of cirrhosis of the liver induced experimentally, Higgins and Stasney (1936) observed that the bone marrow developed normoblastic hyperplasia. Fiessinger, Dupuy and Laur (1937) believe that such a normoblastic hyperplasia is not due to repeated hæmorrhages. Révol (1938) studied the marrow and found an increase of normoblasts with proerythroblasts which were very

similar in appearance to megaloblasts. Benhamou and Nouchi (1937) distinguish three types :—

(1) Macrocytic anæmia with early and late normoblasts in the marrow.

(2) Microcytic hypochromic anæmia with micronormoblastic reaction in the marrow.

(3) Erythrocytosis with normoblastic reaction in the marrow.

Roversi and Tanturri (1935) recorded moderate but inconstant normoblastic hyperplasia. In our own cases we have invariably found hyperplasia of erythropoiesis. Falzoj (1939) noted that in diseases of the liver the proerythroblasts and micronormoblasts were increased and showed premature nuclear maturation, and that the number of mitoses of the basophilic and polychromatic normoblasts was also increased. Megakaryocytes were reduced and so were hæmocytoblasts and hæmohistioblasts. Myeloblasts and promyelocytes showed disturbances of maturation. Oria, Ramos and Tranchesi (1938), in cirrhosis of the liver, reported normoblastic hyperplasia with large and small forms, but without megaloblasts. Sack (1940) investigated cases of chronic arsenical poisoning with cirrhosis of the liver, which occurs in vintners, and found increases of erythroblasts and reticulum cells. Similar observations have been made in alcoholic hepatic cirrhosis. Our observations showed that there is some connection with the anti-pernicious anæmia factor or with some other hepatic factor of fundamental importance for hæmopoiesis.

Case 69. B. E., a farm labourer of 36 years, a chronic alcoholic, had had cholelithiasis in 1934, and in 1937 developed tremors, a hard enlargement of the liver, urobilin and indican in the urine, lassitude and pain in the calves. When examined the liver edge was three fingers' breadth below the costal margin, and hard. Urine: urobilinogen +++, urobilin +. Serum bilirubin direct +, indirect 0.8 mg %. Alcoholic polyneuritis, no ascites. Gastric juice: no hydrochloric acid, with general hyposecretion.

BLOOD. R.B.C. 4.1 millions, Hb 86.4% = 13.9 g %, C.I. 1.05, W.B.C. 6,060; basophils 1%, eosinophils 1.5%, stab forms 0.5%, segmented polymorphs 57%, lymphocytes 33.5%, monocytes 6.5%. Sedimentation rate (Westergren) 4–10 mm. (1 and 2 hr.). Takata-Ara reaction positive, blood cholesterol 99 mg %, serum protein 7.76 g %, albumen-globulin ratio 55 : 45.

STERNAL MARROW. Proerythroblasts 1, early basophilic normoblasts 5.5, normoblasts 52 per 100 white cells; myeloblasts 1%, promyelocytes 2.5%, semimature myelocytes 4.5%, mature myelocytes 6.5%, metamyelocytes 13.5%, stab forms 13.5%, segmented polymorphs 26.5%, eosinophil myelocytes 2.5%, eosinophil metamyelocytes 3%, eosinophils 2.5%, lymphocytes 16.5%, monocytes 1%, megakaryocytes 1%, plasma cells 4%, endothelial and reticulum cells 3%.

In this case there was considerable hyperplasia of erythropoiesis, also involving the early basophilic normoblasts and with an increase of plasma cells. There was no definite myelocytic reaction.

Case 70. B J., a man of 54 years, who had always been rather stout,

The ascites demanded frequent drainage by paracentesis at the rate of about 10 litres a month. He had severe *epistaxis and this was followed* by anæmia (Hb. 30% = 4·8 g %, R.B.C. 1·6 millions), responding slowly to treatment. It was thought *that the hæmorrhage was due to deficiency* of prothrombin.

BLOOD. R.B C *5 04 millions, Hb. 83% = 13·3* g % ; *W.B C. 5,000*, eosinophils 3%, segmented polymorphs 68 5%, lymphocytes 21·5%, *monocytes* 7%. *Sedimentation* rate (Westergren) 22–52 mm. (1 and 2 hr.). Serum bilirubin : indirect delayed positive, direct a trace ; blood chole-

blasts 6·75, normoblasts 42·75 per 100 white cells ; myeloblasts 3%,

endothelial and reticulum cells 1%, plasmoblasts 1·25%, proplasmocytes 1 5%, plasma cells 2%.

This was a case of cirrhosis of the liver with severe ascites, in which marked hyperplasia of erythropoiesis with involvement of the early basophilic normoblasts was present in the sternal marrow, persisting even when the anæmia had been corrected. There was a definite plasma cell reaction and a slight myelocytic-metamyelocytic shift to the left, which, however, was no higher than in case 69, which had no ascites. These findings indicate that ascites is a secondary manifestation and not an inflammatory one, as suggested by Tischendorf.

Case 71. R E., a woman of 40 years, had pain in the right hypochondrium in 1933 In 1938, a few weeks before she entered the hospital, she had fever up to 105° F, rigors, attacks of diarrhœa with clay-coloured stools and jaundice When examined, her temperature was 105° F, and she was jaundiced The liver edge was one and a half fingers' breadth below the costal margin The urine contained bile pigments There was a systolic murmur. She produced a purulent sputum An X-ray of the chest showed a lung abscess in the right lower zone The patient ... downhill and died four weeks later The autopsy confirmed the

... ınısocy-
68·5%,

lymphocytes 9%, monocytes 2%, toxic granulation of ... utrophil polymorphs Sedimentation rate (Westergren) 159–162 mm (*1 and 2 hr*) Serum bilirubin direct ++, indirect 2·4 mg %, blood cholesterol 103 mg %, blood urea 11 mg.%, serum protein 6 9 g % albumen-globulin ratio 40 . 60, Takata-Ara reaction positive at the beginning, later negative

STERNAL MARROW Proerythroblasts 0·5, early basophilic normo-

blasts 1·5, normoblasts 7·5 per 100 white cells; myeloblasts 3·5%, promyelocytes 3·5%, semimature myelocytes 3%, mature myelocytes 13·5%, metamyelocytes 13%, stab forms 22%, segmented polymorphs 23%, eosinophil myelocytes 1%, eosinophil metamyelocytes 1%, eosinophils 1%, lymphocytes 7%, monocytes 1%, megakaryocytes 0·5%, lymphoid and phagocytic reticulum cells 2%, plasmoblasts 0·5%, plasma cells 3%.

In this case the number of normoblasts was much reduced in spite of marked jaundice and an increase of bilirubin in the blood (2·1 mg.%) Progressive anæmia developed. In spite of blood transfusions, Campolon and ferrous preparations, the number of erythrocytes fell to 1·85 millions and the hæmoglobin to 36·7% = 5·9 g.% Macrocytosis, which in cases 69 and 70 had been a prominent feature, was not present in case 71. On the other hand, the myelocytic shift to the left was more marked (more than 20% stab forms in the peripheral blood), and a plasma cell reaction was also observed These 3 cases, cirrhosis with, and cirrhosis without ascites, and purulent abscess-forming hepatitis, illustrate that the development of macrocytosis does not depend on the level of bilirubin in the blood, but more probably on the hæmopoietic factor. In the case with the highest figure of bilirubin, the erythrocytes were normocytic Apart from the inherent faculty of the marrow to regenerate, the hæmopoietic factor also is of importance in the production of hyperplasia of erythropoiesis. In case 71 the marrow was severely damaged by the toxic infection present The myelocytic shift to the left was most marked in the case with the purulent process, not very marked in the case of cirrhosis with ascites, and just present in the case of cirrhosis without ascites The plasma cells were increased in all 3 cases, but there does not appear to be any relationship between them and the red cell precursors The first 2 cases also showed an increase of phagocytic reticulum cells The following case of toxic hepatitis illustrates the blood and marrow pictures in acute and diffuse damage to the liver parenchyma —

Case 72. G H., an apprentice of 17 years, previously had not had any serious illnesses He was admitted with infiltration of the left upper zone of the lung and sputum containing tubercle bacilli In the lower zones there was also infiltration and some areas of collapse An artificial pneumothorax was induced and he improved steadily He suddenly developed a dental abscess of the left upper canine and the first premolar teeth, with much swelling in that region and pyrexia. The administration of prontosil (3 g daily for 5 days) resulted in a reduction of the temperature and a diminution of the swelling He developed jaundice and the liver became tender to palpation The stools were pale and contained no bile pigments The urine contained bilirubin

Blood R B C 4·77 millions, Hb 81% = 13 g %, C I 0·85, W B C 7,850, basophils 1·5%, eosinophils 1% stab forms 10·5%, segmented polymorphs 57%, lymphocytes 19%, monocytes 10·5%, plasma cells 0·5% Platelets 265,000, reticulocytes 1·1% Serum bilirubin direct + indirect 39 mg %

STERNAL MARROW. Proerythroblasts 1, early basophilic normoblasts 1·6, normoblasts 42·3 per 100 white cells; myeloblasts 0·6%, promyelocytes 6·3%, semimature myelocytes 10·6%, mature myelocytes 14·3%, metamyelocytes 16·6%, stab forms 15·3%, segmented polymorphs 10%, basophil cells 0·3%, eosinophil myelocytes 1·6%, eosinophil metamyelocytes 1%, lymphocytes 6·3%, monocytes 1%, megakaryocytes 1·6%, proplasmocytes 2·6%, plasma cells 5·3%, lymphoid reticulum cells 1%, phagocytic reticulum cells 4%.

In this case of toxic jaundice following sulphanilamide medication the erythropoietic portion of the marrow showed hyperplasia, which involved the orthochromic and polychromatic normoblasts, but not the proerythroblasts and early basophilic normoblasts. The myelocytic shift to the left was not marked though quite definite. Plasma cell proliferation was pronounced. The patient made good progress, the jaundice subsided in 10 days.

Another disease which may be mentioned here is infective hepatitis. We have been able to examine 15 patients during a small outbreak. The clinical manifestations were very similar in all the cases. The results of blood and marrow findings of 7 of them are summarized in Table 19. In all cases there was a definite increase of lymphocytes in the blood, the large lymphocytes being affected also. In 4 cases the plasma cells and monocytes were also definitely increased. Table 19 gives the blood pictures, which showed a lymphatic reaction most definitely during the course of the disease; blood examinations were made every 3 to 8 days. One exception is case 6, who 8 days after the blood count quoted in the table showed 9% plasma cells. This was the highest figure of plasma cells seen in our series. Landolt (1942) has observed even more pronounced increases of plasma cells (up to 16%), but Burger (1943) found mostly figures between 2% and 5%. Unlike Holler (1942) and Landolt, we have not observed enlargement of the lymph glands, nor have we seen any definite plasma cell proliferation in the sternal marrow, and therefore we suggest that the plasma cells originate in the lymphatic organs, and not in the bone marrow. The lymphatic reaction was absent in only 1 of the 15 cases, that of a typist of twenty-seven years, who had previously had tuberculosis of the spine. The highest figure for lymphocytes found was 65%. An atypical, particularly persistent case was that of a man of 59 years, in whom the number of lymphocytes was 61%, 11% being large forms. He also showed 2% plasma cells and 4·5% monocytes. The lymphatic reaction subsided in a few cases after about a week, in most cases it lasted 10-14 days, and in a few as long as 3 weeks. A lymphatic reaction, as judged by our observations, seems to be peculiar to virus diseases.

The number of monocytes was definitely increased in cases 1, 3, 4, 6 and 7, invariably more than 10%, with a maximum of 18%. Holler (1942) frequently found very high figures for monocytes

TABLE 19

Blood and Marrow Findings in Infective Hepatitis

	Normal Values	1 B.B.	2 S.F.	3 K.B.	4 K.V.	5 B.L.	6 P.A.	7 G.J.
Blood								
Erythrocytes in millions per c.mm.		4.9	4.5	4.775	5.25	4.8	5.35	5.12
Hæmoglobin in % (100% = 16 g.%)		104	108	90	93	88	122	100
Colour index		1.06	1.2	0.94	0.88	0.91	1.16	1.0
Leucocytes per cmm		4,850	7,300	2,850	5,100	5,000	8,100	6,450
Basophils %		—	1.0	2.5	0.5	—	—	0.5
Eosinophils %		1.0	6.0	4.5	0.5	1.0	0.5	3.0
Stab forms %		2.0	—	3.0	4.0	1.5	1.5	3.5
Segmented polymorphs %		38.0	44.0	23.0	32.0	55.5	28.0	37.5
Lymphocytes %		38.0	42.0	44.0	49.0	38.5	45.0	41.0
Monocytes %		18.0	7.0	17.0	12.0	2.5	13.0	10.5
Plasma cells %		3.0	1.0	6.0	2.0	1.0	8.0	4.0
Blood sedimentation rate in mm. per hour		40/75	32/61	34/72	40/70	22/46	11/32	15/35
Sternal marrow								
Proerythroblasts (per 100 white cells)	0.8	1.0	1.0	1.0	1.6	0.6	0.6	1.5
Early normoblasts (per 100 white cells)	3.2	4.3	2.6	3.3	4.0	1.6	3.0	3.25
Late normoblasts (per 100 white cells)	24.4	33.6	28.6	38.6	35.3	29.3	33.0	39.5
Myeloblasts %	1.2	1.0	1.0	2.0	0.6	0.6	1.0	1.0
Promyelocytes %	2.2	2.3	1.3	3.3	4.3	2.3	2.3	3.25
Semimature myelocytes %	5.4	3.6	5.6	6.0	7.6	7.0	3.6	5.75
Mature myelocytes %	7.2	14.3	12.3	14.6	13.3	13.3	15.0	13.25
Metamyelocytes %	10.2	22.6	17.6	15.0	18.6	20.6	17.6	16.25
Stab forms %	24.0	18.6	22.0	13.3	19.0	19.0	20.3	17.0
Segmented polymorphs %	28.4	12.0	20.0	17.6	21.3	16.3	15.0	16.25
Basophil myelocytes %	0.02	—	0.6	1.3	—	—	—	—
Basophils %	0.02	—	0.6	1.0	0.3	—	—	—
Eosinophil myelocytes %	1.4	1.0	1.0	3.0	1.0	2.3	4.3	2.0
Eosinophil metamyelocytes %	0.8	2.3	1.0	2.3	0.6	1.3	2.0	1.75
Eosinophils %	1.8	3.6	2.3	1.6	0.6	1.3	1.6	1.25
Lymphoblasts %	1.0	—	0.3	0.3	0.3	—	—	0.25
Lymphocytes %	7.6	5.0	6.3	6.3	8.3	5.0	6.0	7.75
Monocytes %	1.4	1.3	0.6	1.0	0.3	1.6	1.0	1.25
Megakaryocytes %	0.8	0.6	1.6	2.0	0.3	1.0	2.0	1.5
Plasmoblasts %	0.2	—	0.3	0.6	0.3	0.3	0.3	0.25
Proplasmocytes %	0.3	0.3	0.3	0.6	0.3	0.6	0.3	0.25
Plasma cells %	0.5	2.3	1.3	2.6	1.0	2.0	1.3	2.0
Lymphoid reticulum cells %	1.0	1.0	0.6	0.6	0.3	1.0	0.6	0.25
Phagocytic reticulum cells %	0.2	1.3	1.3	2.0	1.0	2.3	1.6	0.5
Endothelial cells %	0.8	—	—	—	0.3	0.6	0.3	—
Fat cells %	0.4	—	—	0.3	0.3	—	0.3	0.25

(up to 45%) He regards these findings as evidence of a generalized acute reticulo-endotheliosis, of which jaundice is merely a symptom Burger (1943), who examined a large number of cases, found more than 10% monocytes in only 3 patients. He believes that Holler's

figures are so high because large monocytes and lymphocytes were counted together. The distinction of monocytes and lymphocytes did not present any difficulties in our series of cases. Apart from the usual preparations, peroxidase staining was carried out in every case and the slightly oxidase-positive monocytes were easily distinguishable. More cases of monocytosis were found than by Burger, and like Holler we are inclined to regard them as evidence of a reticulo-endothelial reaction. On the other hand we reject the idea of a reticulo-endotheliosis, because the histological basis for this is quite inadequate. In 86% of his cases, Beiglböck (1944) found more than 5% monocytes.

In our cases the shift to the left of the neutrophils was not as marked as in Burger's cases. His most common finding was 10% stab forms. In 3 of our 15 cases, there was an eosinophilia, the highest count being 5%.

In the sternal marrow we found that every case showed a myelocytic shift to the left, but again the shift was not so pronounced as in the cases described by Burger. Landolt (1942) examined 3 cases by sternal puncture and found normal marrow. Unlike Burger we have not been able to demonstrate definite plasma cell hyperplasia, which we invariably found in simple jaundice and in cirrhosis of the liver. When examining marrow smears, the megakaryocytes appeared to be increased in numbers, but when exact counts were made this was not substantiated. The marrow giant cells were normal morphogically. This tallies with the finding that the platelets in the blood were morphologically and quantitatively normal. The phagocytic reticulum cells were increased, but the maximum number was only 2·3% in case 5 The figure of normoblasts was not raised in every case, and when it was, the increase was only moderate. The blood sedimentation rate was raised in all cases, quite often markedly The level of bilirubin in the serum was increased even in the cases where jaundice was not marked. Contrary to Eppinger's (1937) opinion, and agreeing with Burger, the invariably raised sedimentation rate favours the diagnosis of infective hepatitis

Summary. Sternal puncture enables us to make the distinction between infective and non-infective (e g., cirrhotic) diseases of the liver. The former is characterized by a myelocytic shift to the left. Infective hepatitis demands a special position because of its lymphatico-plasma cellular reaction in the blood and the frequent increase of monocytes In infective hepatitis, normoblastic hyperplasia was not so marked as in cirrhosis of the liver, as observed by sternal puncture. The myelocytic shift to the left favours the theory of the infectious nature of hepatitis In cirrhosis of the liver there was plasma cell proliferation in the marrow without plasma cells in the peripheral blood In infective hepatitis, in spite of a considerable increase of plasma cells in the peripheral

blood, they were not increased in the sternal marrow. This phenomenon favours the theory of the extramedullary origin of the blood plasma cells in hepatitis.

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CHAPTER XV

DISORDERS OF HÆMOPOIESIS IN ENDOCRINE DISORDERS

NAEGELI (1931) was one of the first to point out the importance of the endocrine glands in the formation of blood. There are, however, relatively few records of investigations by marrow biopsy into these problems, though apart from animal experiments this technique should offer the greatest hope for an understanding of the part played by the endocrine glands. De Renzi (1939) collected a series of 32 patients with various endocrine disorders. He noted that in 13 of his cases there were a large number of normoblasts with punctate basophilia. Beer (1942) has described experiments showing the connection between central nervous stimuli and the hormones in leucopoiesis and erythropoiesis.

Thyroid

Deusch (1921), Thaddea (1932), Curschmann (1941), Mansfeld (1943) and many other authors maintain that there is some connection between the thyroid and the bone marrow. Heilmeyer (1933) observed stimulation of erythropoiesis following the administration of thyroid Mansfeld and Sós (1938) isolated a marrow-stimulating substance, which they call myelotropic hormone, and which they state is necessary when hæmopoiesis is stimulated by oxygen-deficiency The myelotropic hormone is also necessary for the production of the anti-pernicious anæmia factor

Markoff (1939), Balo (1939) and Knittel (1940) induced hyperthyroidism in rabbits by giving thyroxin, and found that the bone marrow underwent general hyperplasia and fatty marrow was transformed into cellular marrow. Increases of normoblasts, promyelocytes and myelocytes were marked, but the megakaryocytes did not increase until the 40th to 50th day The reticulum cells were also increased Following marrow hyperplasia the animals developed osteoporosis. In the peripheral blood an increase of erythrocytes was noted, but the number of leucocytes and platelets remained unchanged Thaddea (1932) studied rabbits which had been given thyroxin and found increases of erythrocytes and of hæmoglobin and also of platelets, as well as neutrophilia and lymphopenia. Beer (1942) observed decreases of erythrocytes and of leucocytic reactions to infections in thyroidectomized animals But when a central nervous stimulus was applied to these animals they showed the same changes as normal controls. Beer, therefore, concluded that there was no direct relationship between the thyroid and the nervous-

humoral mechanism of regulation. Tramontano and Scala (1941) noted neutropenia and an increase in lymphocytes and monocytes in experimental hyperthyroidism.

In hypothyroidism in man, anæmia has been recorded by Kleiner and Rényi-Vámos (1938). In myxœdema it is often macrocytic in type, as reported by Naegeli (1931) and Baldridge and Green (1934). Andrus *et al* (1931), Holboll (1936), Sharpe (1937) and Vannotti (1940), recorded anæmia of the Addisonian type in hypothyroidism. It is often possible to correct it by thyroid preparations (Roos, 1902; Eppinger and Walzel 1926). Barengo (1940) gave subcutaneous injections of thyroid hormone and observed an increase of erythrocytes and of hæmoglobin, and leucopenia with monocytosis and eosinophilia. Aplastic anæmia has been observed by Thaddea (1932) after thyroidectomy; by Jaffé (1938) in atrophy of the thyroid; and by Weyeneth (1942) in lymphocytic atrophic thyroiditis. Weyeneth thought that the thyroid had some function in opposition to the action of the spleen. After thyroidectomy fatal aplastic anæmia developed owing to splenopathic inhibition of the marrow.

Jones (1940) examined patients with hyperthyroidism by sternal puncture and found hyperplasia of all three marrow systems, but after thyroidectomy the marrow returned to normal. On the other hand, de Renzi and Lenzi (1939) in 17 patients with hyperthyroidism found erythropoiesis increased at the expense of leucopoiesis In 2 cases of myxœdema he noted increased leucopoiesis In hyperthyroidism, Meulengracht (1932) is the only worker who has found an anæmia of pernicious megalocytic type Markoff (1939) believes that these anæmias are due to chronic gastroenteritis with or without liver impairment, and therefore possibly due to deficiency of the anti-pernicious anæmia factor The frequency of liver impairment in hyperthyroidism has been emphasized by Assmann (1931) Migone (1942) found hyperplasia and a shift to the left of the white cells and a delayed maturation of the red series in hyperthyroidism. We ourselves have seen only 1 case of anæmia in hyperthyroidism —

Case 73. T J, an agricultural labourer, aged 56 years, since June, 1938, complained of lassitude, weakness, loss of weight, attacks of sweating, palpitations and bouts of diarrhœa When examined, he was wasted, had exophthalmos, Stellwag's, Graefe's and Moebius's signs were positive. There was a hard nodule in the thyroid, the size of a walnut The basal metabolic rate was + 60%

BLOOD R B C 3 2 millions, Hb 47 5 = 7 7 g %, C I 0 73, W B C 3,660, basophils 1%, eosinophils 4%, segmented polymorphs 66%, lymphocytes 21%, monocytes 7 5%, plasma cells 0 5%, blood cholesterol 108 mg %, blood urea 24 mg %, alkali reserve 56 5 vols % Sedimentation rate (Westergren) 7–17 mm (1 and 2 hr)

STERNAL MARROW Early basophilic normoblasts 1, normoblasts 26 5 per 100 white cells, myeloblasts 2%, promyelocytes 1%, semi-

mature myelocytes 4%, mature myelocytes 14·5%, metamyelocytes 16·5%, stab forms 14%, segmented polymorphs 24·5%, eosinophil myelocytes 4%, eosinophil metamyelocytes 3%, eosinophils 2·5%, lymphocytes 4%, megakaryocytes 0·5%, plasma cells 2·5%, lymphoid and phagocytic reticulum cells 3%.

This was a hypochromic anæmia with a normal number of normoblasts in the marrow. The anæmia improved rapidly with the Abelin milk-vegetable-vitamin diet, vitamin A, Gynergen (ergotamine tartrate) and Prominal (Bayer). After 2 months the red cells had risen to 4·98 millions, Hb. to 74·5% = 12 g.%, and the leucocytes to 5,260. The hyperthyroid state did not improve correspondingly, and it is quite possible that the anæmia was of the alimentary type and independent of hyperthyroidism. It is certainly necessary to exercise caution when a relationship between anemia and hyperthyroidism is suspected. In our case the symptomatic anæmia was corrected by non-specific medical measures. In most cases of hyperthyroidism we have noted normal figures for erythrocytes, but a slight hyperplasia of erythropoiesis in the marrow. In the following case of hypothyroidism there was slight hypoplasia of erythropoiesis :—

Case 74. W. M., a domestic worker, aged 21 years, had bilateral hæmatogeneous pulmonary tuberculosis. She had symptoms of hypothyroidism, a dry skin, mental dulness, tachycardia, enlargement of the heart. Electrocardiogram: slight myocardial degeneration. Basal metabolic rate −18%.

BLOOD. R.B.C. 4·1 millions, Hb. 86% = 13·8 g.%, eosinophils 1%, stab forms 11% [illegible] cytes 9% [illegible]

STERNAL [illegible] blasts 1·6, [illegible] promyelocyt[illegible] mature myelocytes 12%, metamyelocytes 16%, stab forms 18·3%, segmented polymorphs 11%, eosinophil myelocytes 5·3%, eosinophil metamyelocytes 4·3% [illegible] The hypothyroid state improved readily with Di-iodo-thyrosin (Hoffmann-la Roche) and the basal metabolic rate reached normal levels.

This was a case of hypothyroidism with slight anæmia. Sternal puncture showed slight hypoplasia of erythropoiesis, eosinophilia and a slight myelocytic-metamyelocytic shift to the left.

Since the introduction of the new and very effective treatment of hyperthyroidism with thiouracil (Astwood, 1943; Hadorn and Beer, 1945; Watson, 1946); agranulocytosis has been observed relatively frequently. This is probably due, not to hypofunction of the thyroid induced by the medication, but to a toxic action of the drug. Impairment of hæmopoiesis is much less frequent when less

toxic preparations, such as methyl-thiouracil, are used. Sensitization may also play a part, because agranulocytosis appears more frequently when the drug is used intermittently. Since the introduction of penicillin the prognosis of agranulocytosis from thiouracil has improved tremendously (Beierwaltes and Sturgis, 1946) Repeated blood and bone marrow examinations are necessary to avoid mishaps and to institute treatment of agranulocytosis promptly (Gessler, 1946; Moore, 1946; Sikkema *et al.*, 1946) Prophylaxis of agranulocytosis with folic acid suggested by Daft and Sesell (1943) has not been confirmed (Newman and Jones, 1946)

Parathyroids

Markoff (1939) states that a very cellular but normal bone marrow is found in senile osteoporosis and in hyperparathyroidism.

Pituitary

The pituitary gland influences hæmopoiesis in many ways Like other authors, we found erythrocytosis in 2 cases of Cushing's disease, but in a case of the "forme fruste" this was absent. Beer (1942) considers that erythrocytosis is not due to the disease of the pituitary itself, but to the stimulation of the neighbouring hæmopoietic centres. This is contradicted by the findings of Vollmer *et al.* (1939), who, in hypophysectomized animals, have observed a fall in erythrocytes and hæmoglobin. Tramontano and Scala (1941) also observed a fall of leucocytes, especially of the neutrophils, but to a lesser degree also of lymphocytes and monocytes in experimental animals after excision of the pituitary. Dodds *et al.* (1935) found that rabbits treated with pituitrin develop macrocytic anæmia. Gilman and Goodman (1937) believe this is due to hæmolysis, owing to hydræmia caused by the antidiuretic action of pituitrin Thaddea and Waly (1934) gave the thyrotropic hormone of the pituitary to rabbits and found increases of erythrocytes, platelets, reticulocytes and hæmoglobin, but this was not confirmed by Barengo (1940) McFarlane and McPhail (1937) excised the pituitary in guinea pigs and found no effect on the blood picture, but *injections of hypophysin produced severe anæmia*

De Renzi (1939) examined 4 cases of Fröhlich's syndrome and 1 of acromegaly by sternal puncture. Anæmia is often seen in hypofunction of the anterior lobe, as in Simmonds' disease Markoff (1939), on the other hand, recorded a case of hypofunction of the anterior lobe with eunuchoidism, showing erythrocytosis and normoblastic hyperplasia in the marrow. Sellares (1940) examined a case of chromophobe adenoma of the pituitary and noted slight hypochromic anæmia without any evidence of regeneration, and lymphocytosis and eosinophilia After removal of the adenoma,

anæmia with polymorph leucocytosis developed. We have examined 2 cases of acromegaly and 1 of diabetes insipidus.

Case 75. H. A., a man aged 30 years, fell ill in December, 1942, with a pleural effusion. He was 2 metres in height, weighed 87 kg. and pre-

… %, C.I. 1·4; W.B.C. … *polymorphs* 50%, … rate (Westergren) 16–43 mm. (1 and 2 hr.). Weltmann reaction: 5th tube (0·175% $CaCl_2$) showed heat coagulation. Serum protein 8·75 g.%.

STERNAL MARROW. Proerythroblasts 0·3, early basophilic normoblasts 1·6, normoblasts 31·3 per 100 white cells; myeloblasts 1%, … myelocytes 3·6%, mature myelocytes … *forms* 16%, *segmented polymorphs* … myelocytes 5·6%, eosinophil meta- … lymphocytes 7·3%, monocytes 1%, … lum cells 2%, … cells 0·3%. … was moderate … l plasma cell proliferation in the marrow.

In another case in our series there was also slight anæmia (R.B.C. 3·8 millions, Hb. 78% = 12·5 g.%) with good regeneration (*sternal marrow: proerythroblasts* 1·3, *early basophilic normoblasts* 4·6, normoblasts 42·3 per 100 white cells) and myelopoiesis was normal

A case of diabetes insipidus (Leitner, 1945) showed a myeloid shift to the left as the only abnormality:—

due to sarcoidosis) In June he developed swelling of the parotids, and of numerous lymph glands. Miliary dissemination in the lungs was demonstrated *A piece of the parotid and an epitrochlear gland were* removed and the diagnosis of Boeck's sarcoidosis was later confirmed by

… forms 10%, segmented polymorphs … es 3% Sedimentation rate … *serum protein* 10 g %, albumin-globulin ratio 45 : 55, blood calcium 1? 2 mg %.

STERNAL MARROW. Proerythroblasts 1, early basophilic normoblasts 2·5, normoblasts 25·25 per 100 white cells; myeloblasts 0·75%, promyelocytes 2·25%, semimature myelocytes 2·5%, mature myelocytes 18·25%, metamyelocytes 22%, stab forms 22%, segmented polymorphs 16·5%, eosinophil myelocytes 0·5%, eosinophil metamyelocytes 0·5%, eosinophils 0·5%, basophils 0·25%, lymphocytes 3·5%, monocytes 0·5%, megakaryocytes 1%, plasma cells 1%, reticulum cells 1·5%.

Adrenals

Leucocytosis produced by the injection of adrenalin is well known since the investigations of Frey and Hagemann (1921). Behr (1939), Kienle and Malamani (1940) and Kienle (1943) suggested that it is not merely a mechanical phenomenon from contraction of the spleen but is due to the effect of the hormone on the bone marrow. Behr observed that it did not occur in patients with severe hæmopathies, in which the reactivity of the marrow had become extinct (panmyelopathy). Beer (1942) considered that the effect of adrenalin on the blood picture is linked up with the existence of higher centres of regulation in the midbrain, because the effect of adrenalin fails to occur when the cervical cord is severed (Muto and Dohi, 1935; Heilmeyer, 1942) The part played by adrenalin is not yet fully understood. Imai (1936) perfused the femora of rabbits with a fluid containing adrenalin and noted an increase of erythrocytes and leucocytes in the fluid which emerged from the marrow Fiorentini (1937) reported that adrenalin had an inhibitory action on the development of bone marrow tissue cultures Falta (1927) noted transformation of the bone marrow and Walterhöfer (1921) observed myeloid hyperplasia after adrenalin injections.

There are very few clinical observations on this subject in the literature Gibson (1931) recorded a case of aplastic anæmia with granulocytopenia and a hæmorrhagic diathesis, with improvement lasting for 5 years following repeated injections of adrenalin Stockinger (1933) made similar observations

The importance of the hormone of the adrenal cortex for hæmopoiesis has attracted more attention Experimental resections of the adrenal cortex have produced rather contradictory results Viale (1934) found the erythrocytes and leucocytes increased Thaddea and Albers (1937), in cases where the adrenal had been removed, described considerable increases of erythrocytes and also of leucocytes and hæmoglobin as the result of hæmoconcentration. The administration of cortin or the implantation of adrenal cortex caused a return to normal Borchardt (1929) adrenalectomized cats and found that the expected leucocytosis from infections or from puncture of the midbrain did not occur Muto and Dohi (1935) in rats, La Grutta (1937) in cats, and Beer (1942) in rabbits however, found that after the first 24 hours the animals behaved normally Beer failed to find characteristic changes in the blood picture The immediate bone marrow response seemed to be in abeyance just after operation, but then slowly returned to normal When cortical hormone was given at the same time as the operation the marrow showed the normal rapid regenerative response to infection. This, he argued, indicated the stimulating action of the cortical hormone His findings agree with those of Fiorentini (1937), who demonstrated the

anæmia with polymorph leucocytosis developed. We have examined 2 cases of acromegaly and 1 of diabetes insipidus.

Case 75. H

a pleural effus

and after 40 minutes, 118 mg.%. Blood pressure 105/70 mm. Hg.

BLOOD. %, C.I. 1·4; W.B.C. 6,600; polymorphs 50%, lymphoc rate (Westergren) 16–43 mm. (1 and 2 hr.) Weltmann reaction: 8th tube (0·175% $CaCl_2$) showed heat coagulation Serum protein 8·75 g %.

STERNAL MARROW. Proerythroblasts 0·3, early basophilic normoblasts 1·6, normoblasts 31·3 per 100 white cells; myeloblasts 1%, yelocytes 3 6%, mature myelocytes forms 16%, segmented polymorphs myelocytes 5 6%, eosinophil meta- lymphocytes 7·3%, monocytes 1%, megakaryocytes 0 6%, plasma cells 7%, primitive reticulum cells 2%, phagocytic reticulum cells 1%, endothelial cells 0 6%, fat cells 0 3%.

Eosinophilia was marked in blood and marrow, and there was moderate anæmia with fair regeneration (reticulocytes 3 8%) and plasma cell proliferation in the marrow.

In another case in our series there was also slight anæmia (R B.C. 3·8 millions, Hb. 78% = 12·5 g.%) with good regeneration (sternal marrow: proerythroblasts 1·3, early basophilic normoblasts 4·6, normoblasts 42 3 per 100 white cells) and myelopoiesis was normal.

A case of *diabetes insipidus* (Leitner, 1945) showed a myeloid shift to the left as the only abnormality:—

demonstrated. A piece of the parotid and an epitrochlear gland were removed and the diagnosis of Boeck's sarcoidosis was later confirmed by a biopsy from an inguinal gland. In October, 1943, he developed polyuria (up to 4 litres daily) and polydypsia. The sella turcica was not enlarged.

BLOOD. 3 55 millions, Hb. 80% = 12 8 g.%; W.B.C 11,500, basophils 2%, eosinophils 5%, stab forms 10%, segmented polymorphs 66%, lymphocytes 14%, monocytes 3%. Sedimentation rate (Westergren) 72–108 mm. (1 and 2 hr.); serum protein 10 g %, albumen-globulin ratio 45 : 55, blood calcium 17·2 mg.%.

STERNAL MARROW.

2·5, normoblasts 25·25

myelocytes 2·25%, sem

18 25%, metamyelocytes 22%, stab forms, segmented polymorphs 16 5%, eosinophil myelocytes 0 5%, eosinophil metamyelocytes 0 5%, eosinophils 0·5%, basophils 0 25%, lymphocytes 3 5%, monocytes 0 5%, megakaryocytes 1%, plasma cells 1%, reticulum cells 1 5%

Adrenals

Leucocytosis produced by the injection of adrenalin is well known since the investigations of Frey and Hagemann (1921). Behr (1939), Kienle and Malamani (1940) and Kienle (1943) suggested that it is not merely a mechanical phenomenon from contraction of the spleen but is due to the effect of the hormone on the bone marrow. Behr observed that it did not occur in patients with severe hæmopathies, in which the reactivity of the marrow had become extinct (panmyelopathy). Beer (1942) considered that the effect of adrenalin on the blood picture is linked up with the existence of higher centres of regulation in the midbrain, because the effect of adrenalin fails to occur when the cervical cord is severed (Muto and Dohi, 1935; Heilmeyer, 1942). The part played by adrenalin is not yet fully understood. Imai (1936) perfused the femora of rabbits with a fluid containing adrenalin and noted an increase of erythrocytes and leucocytes in the fluid which emerged from the marrow. Fiorentini (1937) reported that adrenalin had an inhibitory action on the development of bone marrow tissue cultures. Falta (1927) noted transformation of the bone marrow and Walterhöfer (1921) observed myeloid hyperplasia after adrenalin injections.

There are very few clinical observations on this subject in the literature. Gibson (1931) recorded a case of aplastic anæmia with granulocytopenia and a hæmorrhagic diathesis, with improvement lasting for 5 years following repeated injections of adrenalin. Stockinger (1933) made similar observations.

The importance of the hormone of the adrenal cortex for hæmopoiesis has attracted more attention. Experimental resections of the adrenal cortex have produced rather contradictory results. Viale (1934) found the erythrocytes and leucocytes increased. Thaddea and Albers (1937), *in cases where the adrenal had been removed, described considerable increases of erythrocytes and also of leucocytes and hæmoglobin as the result of hæmoconcentration. The administration of cortin or the implantation of adrenal cortex caused a return to normal. Borchardt (1929) adrenalectomized cats and found that the expected leucocytosis from infections or from puncture of the midbrain did not occur. Muto and Dohi (1935) in rats, La Grutta (1937) in cats, and Beer (1942) in rabbits however, found that after the first 24 hours the animals behaved normally. Beer failed to find characteristic changes in the blood picture. The immediate bone marrow response seemed to be in abeyance just after operation, but then slowly returned to normal. When cortical hormone was given at the same time as the operation the marrow showed the normal rapid regenerative response to infection. This, he argued, indicated the stimulating action of the cortical hormone. His findings agree with those of Fiorentini (1937), who demonstrated the*

enhancing effect of cortin for the development and maturation of bone marrow cultures. Lewis (1941) found marrow *hypoplasia in the terminal stages of adrenal insufficiency. Anæmia frequently* occurs in Addison's disease *and it usually responds quite well to* the cortical hormone.

Case 77. G. E., a woman of 51 years, complained of lassitude, weak- [illegible] rs. On examina- [illegible] sted, and X-rays [illegible] Blood pressure 115/70 mm. Hg.

BLOOD. R.B C. 3·4 millions, Hb. 82·1% = 13 2 g.%; W B.C. 5,580; basophils 2·5%, eosinophils 2 5%, segmented polymorphs 53%, [illegible]

Sternal puncture showed, therefore, slight hypoplasia of erythropoiesis, *while the blood showed a normochromic anæmia. After* the administration of Cortigen, sodium chloride (15 g. daily by mouth), Redoxon and Benerva the patient improved and the anæmia disappeared.

With hyperfunction of the adrenals, the red cell count rises (Gunther, 1942) and erythrocytosis may develop.

Diabetes Mellitus

Markoff (1936) examined diabetics by sternal puncture and found a myelocytic-promyelocytic marrow with an increase of the *phagocytic reticulum cells and the storage of doubly refractile* substances. This latter phenomenon has been observed in our experience *only when the ketone bodies were increased in the blood* Schweers (1938) reported a leukæmoid reaction in a case of hyperglycæmic coma, but he did not examine the sternal marrow Kasuga (1938) believes that the diameter of the red cells is large in diabetes, but we could not confirm this. Gubernale (1940) observed an increase of immature red and white cells, which disappeared with insulin treatment. He also recorded slight degrees of *anæmia All we have found is a myelocytic shift to the left and an* increase of the fat-storing cells.

Case 78. H L., a woman of 56 years, had been a diabetic for a year. She had lost about 20 kg in weight lately On examination: Urine sugar 7% = 51·7 g per day, acetone + *Blood sugar 310 mg %*

Blood. R.B.C. 4.8 millions, Hb. 100% = 16 g %; W.B.C. 8,840; stab forms 1·5%, segmented polymorphs 61%, lymphocytes 33%, monocytes 4.5%; sedimentation rate (Westergren) 11–34 mm. (1 and 2 hr.) Serum bilirubin negative. Blood cholesterol 370 mg.%, urea 22 mg %, alkali reserve 50.5 vol.%.

Sternal Marrow. Proerythroblasts 0.5, early basophilic normoblasts 1·5, normoblasts 30 per 100 white cells; myeloblasts 1·5%,

[illegible]

There were thus no blood or marrow changes characteristic of diabetes mellitus. The increase of fat cells in the marrow appears to be the least inconstant feature, as compared with other endocrine disorders.

Sex Glands

Many instances of disturbance of hæmopoiesis from dysfunction of the sex glands have been recorded. *Hypofunction* frequently leads to anæmia. Even chlorosis and idiopathic hypochromic anæmia have been linked with hypofunction of the sex glands. Denecke and Josam (1927) gave ovarian hormone, and Molteni (1929) and Tuffi (1929) gave testicular extract to male animals and observed a reticulocytosis in the peripheral blood. Hoff (1934) reported a case of chlorosis-like anæmia in hypogenitalism, which benefited from the administration of sex gland and pituitary products, and in which the anæmia improved with this treatment. Sellares (1940) observed a case of Fröhlich's syndrome with hypochromic anæmia, eosinophilia and lymphocytosis. In the sternal marrow the granular cells were increased. Esser (1940) described a case of "congenital aplastic anæmia" in a male mongol baby of nine months with atrophy of the testicles. Gonnermann (1938) reported a case of hypochromic anæmia in a youth of seventeen years. The anæmia failed to respond to iron, but improved readily with testicular extract. We have seen a case of severe masculine hypogenitalism (eunuchoidism) probably due to multiglandular disturbances :—

Case 79. O. E., a watchmaker, aged 51 years, has been a diabetic for 10 years. In March, 1943, he became ill with lassitude and a productive

[illegible]

pulmonary tuberculosis with a large right apical cavity and a right-sided

effusion, which improved considerably with pneumothorax therapy. Vital capacity 180 c c.; inspiratory pause 20 seconds. Blood pressure 170/120 mm. Hg Tuberculin reaction: Mantoux 1 in 100,000 negative, 1 in 10,000 positive.

BLOOD. R.B.C. 5·27 millions, Hb. 97% = 15 5 g.%; W.B.C. 8,900; eosinophils 18%, stab forms 1%, segmented polymorphs 45%, lymphocytes 30%, monocytes 5%, plasma cells 1%. Sedimentation rate (West

1, early basophilic normoblasts
s; myeloblasts 1% promyelo-
cytes 0·25%,
metamyelocyt
basophils 0·25
4·25%, eosino
karyoblasts 1
proplasmocytes %, plasma cells 2·75%, primitive reticulum cells 0 5%, phagocytic reticulum cells 1%, fat cells 1·75%.

This was a case of severe hypogenitalism with eunuchoidism, diabetes mellitus and pulmonary tuberculosis with a cavity. There was no anæmia, but the blood picture showed eosinophilia, the cause of which was not found. Apart from eosinophilia, the sternal marrow showed a slight myeloid shift to the left, slight hyperplasia of the megakaryocytic series and increases of the phagocytic reticulum cells and of fat cells.

In hypogenitalism in the female we have seen anæmia improve on hormone therapy:—

Case 80. G. E, a woman of 27 years, of infantile appearance and habits lained of lassi-

%, C I 0 79;
eosinophils 1·5%, stab forms 0 5%, segmented polymorphs 61·5%, lymphocytes 32·5%, monocytes 4%. Sedimentation rate (W
S
10 5
matu
stab
0 5%
mono
1%.

Following treatment with ovarian hormone the patient improved and the blood picture returned to normal This was a case of hypogenitalism with slight anæmia, readily responding to ovarian hormone therapy.

Saurer (1943) published an interesting case; his patient was given Ovocyclin (Ciba) and the amenorrhœa and anæmia were cured, and most surprisingly also the achlorhydria, which must have been due to hormonal influence Feuchtinger (1942) also found that small doses of follicular hormone, Progynon B (Schering), resulted in a rise of the hæmoglobin content

and of the numbers of red and white cells and platelets, while large doses led to anæmia. Tanzi (1941) gave 8 mg. Folliculin to guinea pigs and found increases of reticulocytes and moderate leucocytosis.

Arnold *et al.* (1937) gave large doses of follicular hormone to dogs, and this produced hæmorrhagic thrombocytopenia, anæmia and leucopenia. Post-mortem marrow sections showed increases of the leucopoietic and decreases of the erythropoietic portions of bone marrow. Schrade (1938) confirmed these results in dogs and investigated the reaction of the bone marrow by biopsies. Large doses (50,000 international units) of Progynon produced leucocytosis, which, however, did not reach the severity of a leukæmoid reaction and was mainly due to increases of cells at the intermediate stages of maturation. He could not confirm the findings of Arnold *et al.* (1937), as far as the leucocytes were concerned, but like them he found anæmia and thrombocytopenia. In the marrow the myeloid cells of the intermediate stages of maturation were increased, the erythroblasts were diminished. These marrow biopsy findings agreed with those obtained by autopsy by Arnold *et al.* The white cells showed maturation arrest, and the red, hypoplasia. Feuchtinger (1940), however, could not confirm these results in rabbits or rats. The maturation arrest produced in these animals tended to disappear in spite of the prolonged administration of high doses of synthetic follicular hormone. About six months later marrow insufficiency developed, not directly fatal in itself; death was usually due to peritonitis or cerebral hæmorrhage. Feuchtinger stated that maturation arrest in man also is only transient after high doses of follicular hormone. In the dog, manifestations of overdosage can be prevented by the administration of liver and iron, the liver having a detoxicating action and the iron stimulating the bone marrow. Bareuther and Schabbel (1937), working on dogs, which were given toxic doses of follicular hormone, produced at first thrombocytopenia with a prolonged bleeding time and a decrease of reticulocytes, later also anæmia and leucopenia. They never observed panmyelopathy, but maturation arrest occurred in many cases. Bokelmann (1937) described a human case of panmyelophthisis after the administration of 140 mg. œstroradiolmonobenzoate. Florentin and Binder (1940) found many lymphocytes with Kurloff-bodies in the lymphatic organs of guinea pigs which had been given folliculin and testoterone. Tramontano and Scala (1941) castrated rabbits and noted leucocytosis in the males and lymphocytosis in the females. Varady (1940) could not find any significant blood changes in infants with gonorrhœa treated by follicular hormone. The majority of investigations favour the view that the ovarian factor which is capable of influencing hæmopoiesis is probably the follicular hormone

effusion, which improved considerably with pneumothorax therapy. Vital capacity 180 c c.; inspiratory pause 20 seconds. Blood pressure 170/120 mm. Hg Tuberculin reaction: Mantoux 1 in 100,000 negative, 1 in 10,000 positive.

BLOOD. R.B C. 5 27 millions, Hb. 97% = 15·5 g.%; W.B C. 8,900; eosinophils 18%, stab forms 1%, segmented polymorphs 45%, lymphocytes 30%, monocytes 5%, plasma cells 1%. Sedimentation rate

., early basophilic normoblasts
, myeloblasts 1%, promyelo-

proplasmocytes 0 5%, plasma cells 2·75%, primitive reticulum cells 0·5%, phagocytic reticulum cells 1%, fat cells 1·75%.

This was a case of severe hypogenitalism with eunuchoidism, diabetes mellitus and pulmonary tuberculosis with a cavity. There was no anæmia, but the blood picture showed eosinophilia, the cause of which was not found. Apart from eosinophilia, the sternal marrow showed a slight myeloid shift to the left, slight hyperplasia of the megakaryocytic series and increases of the phagocytic reticulum cells and of fat cells.

In hypogenitalism in the female we have seen anæmia improve on hormone therapy:—

W.B.C. 7,000; eosinophils 1·5%, stab forms 0·5%, segmented polymorphs 61·5%, lymphocytes 32 5%, monocytes 4%. Sedimentation rate (Westergren) 5–16 mm. (1 and 2 hr).

STERNAL MARROW Early basophilic normoblasts 1, normoblasts 10·5 per 100 white cells, myeloblasts 0 5%, promyelocytes 1·5%, semimature myelocytes 1%, mature myelocytes 5%, metamyelocytes 9 5%, stab forms 22%, segmented polymorphs 27%, eosinophil myelocytes 0·5%, eosinophil metamyelocytes 2%, eosinophils 1%, lymphocytes 26%, monocytes 1%, megakaryocytes 1·5%, plasma cells 0·5%, reticulum cells 1%.

Following treatment with ovarian hormone the patient improved and the blood picture returned to normal This was a case of hypogenitalism with slight anæmia, readily responding to ovarian hormone therapy.

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20

in Pregnancy

5 F R 20 (a) 6	(b) 7	6 K M 24 4	7 J J 22 5	8 M A 31 6	9 N P 29 6	10 N C 28 7	11 F M 24 8
3.6	4.1	3.3	4.14	4.25	3.9	4.0	4.3
87	88	68	88	82	80	60	85
6,100	7,300	8,400	9,020	7,900	9,120	8,700	8,750
1.0	0.5	—	—	0.5	0.5	—	—
6.0	4.5	—	—	1.5	0.5	1.0	3.5
2.0	3.0	—	2.0	—	2.5	5.0	3.5
49.0	50.0	74.0	66.0	70.0	65.0	57.0	61.0
37.0	35.0	15.5	28.0	21.5	28.5	32.0	30.5
5.0	7.0	9.5	3.0	3.5	3.0	5.0	2.5
2.0	1.5	1.0	1.0	1.3	2.0	1.5	2.75
4.6	5.75	2.0	1.3	4.6	6.25	2.0	6.75
30.3	38.25	33.5	38.8	34.6	35.75	34.75	37.0
1.0	0.5	0.5	0.3	1.0	1.25	0.5	1.5
4.6	5.25	4.0	1.6	2.6	4.0	2.75	5.75
7.3	8.0	5.5	3.6	9.3	11.0	3.25	13.25
12.0	18.75	8.5	14.0	15.0	13.75	23.5	14.5
19.3	22.25	12.0	11.0	18.3	15.5	15.25	18.25
19.6	23.25	24.0	11.6	21.6	23.5	16.25	17.75
15.0	13.0	25.5	37.3	18.0	18.5	20.0	15.0
2.0	1.5	2.0	0.6	2.3	3.0	1.75	2.25
3.0	1.5	2.0	0.3	2.0	1.25	0.5	1.75
1.6	1.0	2.0	2.3	1.6	1.75	1.5	2.0
0.6	0.25	—	—	1.0	—	1.0	0.5
9.3	4.0	7.0	10.6	6.6	3.25	2.0	4.5
1.0	0.5	1.0	1.3	1.0	0.25	0.5	0.25
1.0	0.5	0.5	0.8	1.3	0.5	0.5	0.25
2.3	1.75	2.0	1.3	4.6	1.0	5.0	2.5
2.0	0.5	0.5	1.0	1.3	1.0	0.25	0.5
1.6	0.25	0.5	1.0	1.0	0.5	0.5	0.5
0.6	0.25	0.5	0.6	1.0	—	—	—
0.3	—	—	—	1.0	—	0.5	—

(3) Pernicious anæmia of pregnancy, already discussed in the section on pernicious anæmia.

(4) Hæmolytic anæmia of pregnancy, a very rare disease, described by Schumann (1933) and Alder (1939).

As far as iron-deficiency anæmia is concerned, it is worth remembering that the fœtus obtains his iron from th[illegible]
iron, which is easily split o[illegible]
the maternal hæmoglobin [illegible]
is no destruction of maternal erythrocytes in the placenta The daily requirement of iron is estimated at 15 mg. by Guggisberg
(19[illegible]

TABLE

Sternal Marrow

Case number and name Age in years Stage of pregnancy in months		1 F J 24 2	2 A M 31 3	3 H A 28 3	4 M O 30 4
Blood count					
R.B.C in millions per cmm.		*4·61*	*4·5*	*4·3*	*4·9*
Hæmoglobin in % (100% = 16 g.%)		85	87	78	89
Leucocytes per cmm.		7,140	6,250	7,200	8,850
Basophils %		0·5	—	1·0	1·0
Eosinophils %		2·0	3·5	1·0	1·0
Stab forms %		*1·0*	*0·5*	*1·5*	*3·0*
Segmented polymorphs %		70·5	63·0	59·0	67·0
Lymphocytes %		21·5	28·0	30·0	21·0
Monocytes %		4·5	5·0	7·5	7·0
Myelogram					
Proerythroblasts	per 100 white cells	*2·75*	*1·75*	*2·0*	*1·3*
Early basophilic normoblasts	per 100 white cells	*5·25*	*4·5*	*5·25*	*3·6*
Late normoblasts	per 100 white cells	29·75	30·75	32·25	38·0
Myeloblasts %		0·75	1·0	0·5	0·3
Promyelocytes %		3·25	3·75	2·25	3·6
Semimature myelocytes %		*7·5*	*9·25*	*10·0*	*5·3*
Mature myelocytes %		10·25	14·0	15·25	14·6
Metamyelocytes %		20·0	18·25	21·0	16·0
Stab forms %		20·5	15·0	14·5	17·3
Segmented polymorphs %		15·75	20·75	21·5	18·0
Eosinophil myelocytes %		*1·0*	1·25	0·5	3·0
Eosinophil metamyelocytes %		1·5	1·0	0·5	2·3
Eosinophils %		1·5	1·75	1·75	2·6
Basophils %		0·5	0·25	0·25	0·6
Lymphocytes %		8·0	6·75	8·0	5·0
Monocytes %		1·25	2·0	0·5	0·6
Megakaryocytes %		0·75	1·0	0·5	2·0
Plasma cells %		2·5	2·5	1·25	3·0
Primitive reticulum cells %		0·5	1·75	0·5	1·0
Phagocytic reticulum cells %		1·0	0·5	1·0	1·3
Endothelial cells %		1·0	0·5	0·25	0·3
Fat cells %		0·5	0·5	–	0·3

ANÆMIA OF PREGNANCY

In the human, the anæmia of pregnancy is of considerable importance. *Schneider (1924).* Kuhnel (1926) and Esch (1932) assessed its frequency as high as 50% of all pregnancies. Guggisberg *(1941) discussed its development and put forward* the following classification :—

(1) Normochromic *anæmia of pregnancy* *Hansen (1938) terms* this disorder "simulated anæmia" and Schultz (1935) "pseudo-anæmia," while Gram (1936) regards it as a *physiological anæmia*

(2) Hypochromic anæmia, which is essentially an iron-deficiency anæmia.

on the basis of a myelogram. In our cases 1, 2, 6 and 7, the hyperplasia only affected the normoblasts (Fig. 188), in other cases it also concerned the granuloblasts, amongst which large cells, similar to the giant neutrophils, were observed.

The highest figure of normoblasts was 42, the highest for early basophilic normoblasts 5·75 per 100 white cells. Contrary to Daniachij, we have not seen megaloblasts. Callender (1946) examined the sternal marrow of 19 healthy pregnant women and found a slight hyperplasia in the late weeks of pregnancy and early in the puerperium.

Summary. Sternal marrow biopsy has so far not contributed towards an advance in our knowledge of the place of the endocrine glands in hæmopoiesis, but in some cases valuable conclusions could be reached In pregnancy an increase of erythroblasts was seen frequently, which was rarely well marked.

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Hansen (1938), Pitts and Packham (1939), Markoff (1939), Pignoli (1942). In 10 pregnant women Daniachij found megaloblasts, Hansen saw an increase of normoblasts, but Pitts and Packham did not find any differences between the marrow of pregnant and non-pregnant women. Markoff considers that the marrow shows a reaction of pregnancy consisting of an increase of erythropoiesis with the formation of normoblastic nests, early basophilic normoblasts and an increase of mitotic figures. In the second month many large early normoblasts can be observed, and Markoff regards this as particularly characteristic. In the granulocytic series he observed anisocytosis of the promyelocytes, often with giant forms, especially prominent halfway through pregnancy. From the sixth month onwards he often noted marrow eosinophilia, indicating hypersensitivity. The plasma cells are also increased according to Markoff, but the reticulum cells are only increased when pathological conditions prevail. Pignoli found a generalized cellular hyperplasia

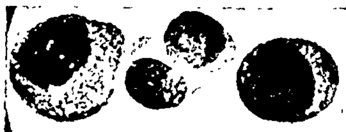


FIG. 188 Sternal marrow in the fifth month of pregnancy: normoblastic hyperplasia with early basophilic normoblasts. (×1,400)

during pregnancy and the first day of the puerperium The proportion of white and red cells was increased in favour of the reds, and there were large erythroblasts and myelocytes and the number of mitoses was increased. Markoff drew up the following scheme for marrow changes in pregnancy:—

Second month . no change among the normoblasts, a few early basophilic normoblasts, anisocytosis of proerythroblasts

Third month: groups of early basophilic normoblasts, many mitoses, and commencing reticulum cell reaction

Fourth month very cellular marrow with generalized hyperplasia and increase of mitoses.

Sixth month · normoblastic foci, many early basophilic normoblasts, marked anisocytosis of promyelocytes, eosinophilia, peak of plasma cell proliferation

Our findings in 11 cases are summarized in Table 20 The reaction of pregnancy shown by the sternal marrow in all our cases was much less marked than was suggested in the literature. It therefore appears that the figures and results quoted from the literature require re-examination and confirmation The marrow hyperplasia certainly is not of such a degree as to allow diagnosis

CHAPTER XVI

THE EFFECTS OF RADIUM AND X-RAYS ON BONE MARROW

Wegelin (1930) describes anæmias of an aplastic type developing as the result of irradiation. Heineke (1905) states that changes may occur as early as the third day. Hyperplasia develops at first and is then followed by aplasia. Wegelin (1930) and Markoff (1936) described an increase of the phagocytic reticulum cells, also seen by Domagk (1928), who noticed karyorrhexis in his experimental animals within a few hours of exposure.

Wünsche (1938) found that high doses of X-rays causes marrow atrophy in rabbits. The white cells were more sensitive to irradiation than the red cells. Marrow and blood monocytosis, he believes, indicate that the damage caused by the rays will be overcome. However, Bauer (1940) found that the early forms of granulocytes were no more sensitive to X-rays than the erythroblasts and that the myeloblasts were particularly ray-resistant. Langendorff and Papperitz (1939) gave doses of 400 r to white mice. They found that after an initial fall there was a marked increase of marrow cells, which eventually gave place to decrease. Later, a second, and later still, a weaker third wave of regeneration became apparent. After 100 days the marrow returned to normal. Engelbreth-Holm (1937) observed leucopenia and especially granulopenia in animals as the early signs of damage from irradiation Panmyelophthisis sometimes occurred later. Feller (1937) exposed rats to radium. While the number of leucocytes fell, myelopoiesis showed signs of regeneration Gregori (1939) found that higher doses (30,000 r.) killed the cells in bone marrow tissue-culture preparations, but much smaller doses, such as 2,000 r , produced heavy damage in the living organism Töppner (1941) gave total body baths to rats and noticed that half an hour later changes in the marrow were obvious. Small doses produced a slight shift to the left and large ones a shift to the right with a decrease of parenchymatous cells. The most radiosensitive cells were the granulocytes, less so the erythroblasts and the megakaryocytes, in that order The reticulum showed only very slight sensitivity Partial irradiations only produce slight changes in the exposed parts, and the untreated portions of marrow are not affected, except by showing increased regeneration The immediate reaction (in the first half-hour) is karyorrhexis (after a dose of 600 r –1,200 r), and the more remote reaction consists of disturbances of maturation, such as hypersegmentation and

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and also Gingold (1939). In view of this hypoplasia of granulocytopoiesis and erythropoiesis we reduced the dosage of rays, or increased the intervals between sessions of treatment. Nordenson (1935) studied a case of Hodgkin's disease of the mediastinal glands treated by irradiation. He found the marrow very poorly cellular with many plasma cells, few myeloid cells and few normoblasts In our case, the reduction in the early cells of the myeloid series was remarkable, while the number of segmented polymorphs was definitely increased (54%). This observation has been confirmed by Florentin and Binder (1940). In 30 cancer cases treated by irradiation to the thorax, the segmented polymorphs were increased at the expense of the more primitive cells. The number of myelocytes fell to 30% after 50 r., to 7% after 600 r, and to zero after 1,200 r. The number of erythroblasts fell in a parallel fashion to 15%, 5% and zero, respectively. The monocytes and eosinophils appeared to be less affected than the neutrophils. Plasma cells were increased. After about 3 weeks the marrow began to show signs of returning to normal. Denstad (1941) suggests that regeneration originates from the mesenchymal cells. Osgood and Bracher (1939) studied the sternal marrow during 7 days after the usual therapeutic dosage of irradiation. They found the lymphocytes decreased (but Florentin and Binder (1940) observed lymphocytosis) and mitotic and amitotic processes were inhibited. There was no difference between the marrow from healthy subjects and from patients with leukæmia. Smaller doses given repeatedly have the same effect as the sum total of single doses In our own cases we did not see decreases of lymphocytes, but the number of mitoses fell profoundly, in Case 81 there were no mitotic figures after irradiation. We believe that sternal puncture can be used to detect early damage of the marrow due to X-rays, and therefore should be used in special cases Meller, Gottlieb and Brauner (1938) also recorded cases where sternal marrow biopsy was more helpful than a blood count In a case of myeloid leukæmia with only 2,000 white cells in the peripheral blood, they were able to give a favourable prognosis based on a relatively good marrow picture In a case of carcinoma of the rectum, the poorly cellular marrow indicated a poor prognosis in spite of a normal blood picture Further development in both cases justified the prognosis based on the myelograms.

Summary. Exposure to X-rays may lead to considerable local damage of the bone marrow The myelocytes are affected, and so are the erythroblasts and the megakaryocytes Cases which have been subjected to heavy dosage may develop marrow aplasia and reticulum cell proliferation Regeneration is possible, even in such severe cases, but further exposure to the rays is undesirable when the marrow is poorly cellular The bone marrow picture

increase in size of nucleus and cytoplasm. The number of mitoses is reduced temporarily. When regeneration sets in occasional pathological mitotic figures are seen as evidence of the dissociation *of nucleus-cytoplasmic division, and binuclear cells may result.*

Schulten (1937) observed an increase of plasma and other reticulum cells in 1 case. Brauner and Gottlieb (1939) made serial sternal marrow biopsies 8 minutes, and 8, 24 and 72 hours after irradiation. They found that small doses had a stimulating action and accelerated maturation, but larger doses proved inhibitory. Besides Wegelin, Markoff, Schulten and Brauner and Gottlieb, whose investigations have been quoted, Piechl (1942) made systematic observations after irradiation with X-rays, and he found changes in the leucocytes similar to those seen in pernicious anæmia, namely, hypersegmentation and the formation of giant neutrophils. Two cases were treated with 30,000 r. There was swelling of the segments of the polymorphonuclears in the peripheral blood and early segmentation of the myelocytes in the sternal marrow. In these cases the results were due to direct local irradiation, because the changes seen occurred only when the sternum was exposed to the rays. We have made observations on the sternal marrow and on the peripheral blood only in pathological cases :—

Case 81. C. E., a man of 60 years, had complained of pain in the [illegible] oma of the [illegible] biopsy. [illegible] ; W.B.C. [illegible] rphs 75%. [illegible] moderate [illegible] 0.5% stab [illegible] 8 [illegible] ular. Normoblasts 2·5 per 100 white c[illegible] ocytes 1%, semimature myelocytes 1·5%, mature myelocytes 4%, metamyelocytes 5·5%, stab forms 8%, *segmented polymorphs* 54%, lymphocytes 9%, monocytes 1%, megakaryocytes 0·25%, endothelial cells 0·5%, plasma cells 5·5%, lymphoid and phagocytic reticulum cells 11·5%. No eosinophils in a count of 400 cells Reticulocytes 0·5%.

Intensive irradiation was followed by hypoplasia of erythropoiesis with 2·5 normoblasts, absence of mitoses and a low reticulocyte count. In contrast to this, *prior to treatment,* figures for erythroblasts were only slightly decreased (early basophilic normoblasts 0·25, normoblasts 18·5) Reticulum cells were markedly increased (plasma cells 5·5%, *lymphoid and phagocytic reticulum* cells 11·5%). This, of course, might have been only relative, because the marrow was very poor in myeloid cells. All the same, the increases *of phagocytic reticulum cells and plasma cells* were definite and our findings agree with those of Wegelin, Markoff

CHAPTER XVII

THE DEMONSTRATION OF PATHOGENS IN STERNAL MARROW

SEYFARTH (1923) used his original method of trephining the sternum to demonstrate malarial plasmodia, but before him Fraenkel (1903) had attempted to isolate pathogenic organisms of various diseases from the marrow at post-mortem. Many pathogens can be more readily demonstrated in the marrow than in the peripheral blood and other organs. Storti and Filippi (1937) believe that this is because the organisms are trapped in the reticulo-endothelial cells of the marrow where they are largely destroyed and therefore cannot always be cultivated. Other authors maintain that the slower rate of blood flow in the bone marrow and the consequent concentration of the organisms are the reasons for their comparatively easy demonstration. We believe that the marrow reticulum cells attract the pathogens initially by bacteriotaxis, and that their destruction and lysis occur later. Thus the organisms are readily cultured from the marrow unless bacteræmia has ceased and no new organisms reach the marrow, or unless the organisms already present have been damaged.

In *malaria* the parasites have been demonstrated by sternal puncture by Kassirsky (1934), Young and Osgood (1935), Benhamou, Nouchi and Bardenat (1937), Schretzenmayr (1938), Storti (1938), Armentano and Bentsath (1940), Lamy (1940) and Magyar (1942). The last five workers have observed parasites in the marrow even when they were absent from the blood. Seyfarth (1923), Picena (1937), Schretzenmayr and Lancaster (1938) and Parise and Lucrezi (1942) state that the marrow contains more plasmodia than the blood. Storti (1938) considers splenic puncture is far superior to marrow puncture in those chronic cases which have had several courses of treatment. The parasites often remain only for a short time in the marrow. Quattrin (1941) injected blood containing malarial parasites intrasternally and found that they disappeared rapidly from the sternal marrow. Schoch (1935) found that plasmodia were not more numerous in the marrow than in the blood. Schulten (1937) describes a case of malignant malaria in which the blood contained only scanty plasmodia and the marrow findings were negative.

In *Leishmaniasis*, sternal puncture is particularly helpful in the diagnosis and much safer than splenic puncture. Positive findings have been reported by Pianese (1905), Kassirsky (1934; in cases of infantile Kala-Azar), Benhamou, Nouchi and Bardenat (1937), Giraud and Gaubert (1937; in 15 of 22 cases), Lorando (1937;

is characterized by an increase of segmented polymorphs at the expense of the myelocytes and by a decrease of mitoses. Plasma cells and phagocytic reticulum cells are increased. Sternal puncture can detect damage to the marrow by rays fairly early. It is, therefore, suggested that in the control of radiotherapy, observations of the sternal marrow by biopsy are of great value, and should be used in conjunction with blood pictures.

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(1937), Giraud and Gaubert (1937;

in every one of his cases), Schretzenmayr and Lancaster (1938), Storti (1938), Bartsocas (1939), Botzaris (1939), Kowalzig (1940) and Redondo (1942). Though the clinical diagnosis may be made on account of splenomegaly, anæmia with the peculiar colour of the skin and leucopenia *alone in the absence of leishmania in the* blood, a positive finding in the sternal marrow makes the diagnosis certain.

Schretzenmayr (1938) and Lamy (1940) reported positive findings in cases of *filariasis. Lamy found the embryo of certain nematodes* and also microfilaria in the sternal marrow. Lamy considers that sternal puncture may be helpful in the recognition of *sleeping sickness* and other diseases *caused by trypanosomes.*

In non-tropical disease, bone marrow culture has been used by Fraenkel (1903) and Debré *et al.* (1935). In cases of *Malta fever* (Brucellosis), Signorelli (1935) invariably found brucellæ, but Storti (1938) found them only in 4 of his 8 cases.

Typhoid bacilli have been demonstrated post-mortem by Fraenkel (1903) from the marrow. Gerbasi (1925), Debré *et al.* (1935), Storti and Filippi (1937), Baserga and Barbagallo (1938), Ott (1938), Storti (1938), Canova (1939), Franza and Colarusso (1939), Hertel (1939), Bertola (1940), Körnig (1940), Lamy (1940), Landau, Bauer and Gryfenberg (1940) and Líng *et al.* (1940) demonstrated typhoid bacilli more frequently in the marrow than *in the blood. Domenighini (1939) and Hertel (1939) found that* sternal puncture failed to prove helpful in particularly mild cases. Baserga and Barbagallo (1938), Ott (1938) and Canova (1939) found that the diagnosis could be made quite frequently on the 8th to 10th day of the disease, when the blood culture was negative *and agglutination tests were only weakly positive. Sprenger (1941)* states that bacteriological diagnosis by examination of the blood and the fæces is positive in 46% of the cases, but positive in 75% by sternal puncture. Franza and Colarusso (1939) collected more than 200 cases of typhoid. Blood culture was positive in 70 and negative *in 141 cases, but culture from sternal puncture material* was positive in 103 and negative in 108 cases. Most positive results were obtained when the specimens were taken during the febrile period, *i.e.*, the first 2 weeks. In 2 cases of *paratyphoid B fever*, Ott (1938) found the organism by marrow culture. In a case of paratyphoid B. septicæmia we obtained a negative result.

Barbagallo (1938) and Storti (1938) examined cases of *subacute bacterial endocarditis* and obtained positive results by sternal puncture. In some cases, positive marrow findings preceded positive blood cultures. In 3 patients with *streptococcus viridans septicæmia* we obtained only one positive marrow culture. *Henning* and Keilhack (1939) studied cases of *hæmolytic streptococcal septicæmia* and *Storti (1938) cases of streptococcal septicæmia*, and they obtained positive marrow cultures. Sternal marrow

cultures gave negative findings in *acute rheumatic fever* (Leitner, Table 21), *diphtheria* (de Filippi, 1939), and *erysipelas* (Bertola, 1940). Cattaneo (1940) investigated 102 patients with *lobar pneumonia* by sternal marrow cultures and found pneumococci grew from the blood and marrow in 18 cases. The organisms remained in the blood and the marrow up to the time of death, or until improvement set in and pyrexia subsided. In 2 cases there was a negative blood, but a positive marrow culture. In most of the cases aged forty years or more, bacteræmia indicated a fatal prognosis. Bertola (1940) also reported positive cultures in cases of lobar pneumonia. Ling *et al.* (1940) observed a case of *staphylococcal septicæmia* which showed a positive marrow culture at 24 hours, but a positive blood culture only after 48 hours.

TABLE 21

Results of Sternal Marrow Cultures

Disease	Number of cases	Blood culture		Marrow culture		Total
		Positive	Negative	Positive	Negative	
1 Non-tuberculous						
Strep. viridans septicæmia	3	2	1	1	2	3
Subacute bacterial endocarditis	4	—	4	—	4	4
Rheumatoid arthritis	5	—	5	—	5	5
Erysipelas	3	—	3	1	2	3
Paratyphoid-B. septicæmia	1	1	—	—	1	1
Multiple abscesses of lungs and liver	1	—	1	—	1	1
Lung abscess	1	—	1	—	1	1
Panmyelopathy	5	—	5	—	5	5
Cholecystitis	1	—	1	—	1	1
Cavernous sinus thrombosis	1	—	1	—	1	1
2 Boeck's sarcoidosis	9	—	9	—	9	9
3 Hæmatogenous tuberculosis	54	—	—	1	53	54
Total	88	3	31	3	85	88

Debré *et al* (1936), Bezançon, Braun and Meyer (1937) and Bernabó-Silorata and Saita (1938) found positive marrow cultures in cases of *tuberculosis* when blood cultures were negative, especially in miliary dissemination. Bonezzi and Ferrari (1938), Leitner and Conradin (1940) and Cremer and Gewecke (1943), on the other hand, usually found negative marrow cultures. Bock (1939) investigated various diseases by comparing cultures from arterial and venous blood and from the marrow. He found that arterial blood gave positive results most consistently. Marrow culture was hardly

superior to venous blood culture. Together with Conradin (1940), we have reported a series of 38 patients with hæmatogenous spread of tuberculosis. In order to study the question of tuberculous bacteræmia, we arranged simultaneous blood and marrow cultures, planted on Löwenstein media. The only positive marrow culture in the series came from a patient with secondary miliary spread of tuberculosis. Although we have studied other cases since then we have not obtained another positive marrow finding. In 25 cases of non-tuberculous disease, mainly comprising virulent infections, we have obtained two positive marrow cultures.

Our results were not convincing and did not produce any evidence that marrow culture was superior to blood culture in coccal infections. The number of non-tuberculous cases was small. In the series of 25 cases, we obtained a positive marrow culture on only two occasions, once in streptococcus viridans septicæmia and once in a case of erysipelas at the height of the infection. Blood cultures were positive in 1 case of streptococcus viridans septicæmia, but negative in erysipelas. In another case of streptococcus viridans septicæmia and in a case of paratyphoid B. septicæmia, blood culture was positive, but marrow culture was negative. In the cases of tuberculosis there was only one positive marrow culture, while all blood cultures were negative. We came to the conclusion that marrow culture offered very little better hope of *demonstrating the organisms than blood culture.* Koch's bacilli are [illegible] degree, and the [illegible] this method. [illegible] blood culture *in tropical diseases, such as malaria, kala-azar,* Malta fever, filariasis and in the typhoid-paratyphoid group. In septic infections, superiority of marrow culture is not proven, or is only very slight and of no practical importance. It is, however, well worth trying in doubtful cases. *As far as tuberculosis is concerned,* the advantages of marrow cultures are not proven and are of no practical importance.

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CHAPTER XVIII

METABOLISM OF MARROW AND BLOOD CELLS

ACCORDING to Schretzenmayr and Bröcheler (1936) and Orr and Strickland (1938) the metabolic activity of *marrow cells is very high. Activity of marrow cells is most marked in the primitive cells.* By using Warburg's method, Schretzenmayr and Bröcheler obtained a value of 18 cmm of O_2 per ml. of sternal marrow per hour. With a hypoplastic marrow the figures were low, while in marrow hyperplasia they were higher. Bréza (1926) also used Warburg's method and found values of 0·13 cmm. per gram per minute for human bone marrow. *Bock and Felix (1939, 1940) state that the oxygen consumption* depends on the degree of cellularity. Soffer and Wintrobe (1932) found *that the oxygen consumption and the* faculty of glycolysis of the granulocytes are greater than those of the lymphocytes. The former are similar to malignant cells and the latter similar to mature tissue cells, as far as their metabolism is concerned. This agrees with the findings of Bungeler (1932) and Bossa (1937). Myeloid leukæmia cells showed a type of metabolism similar to malignant cells, but unlike cancer cells, *lymphatic leukæmia cells showed no abnormality of oxygen* consumption and of anaerobic glycolysis Myeloblasts and lymphocytes showed *the same type* of metabolism. Therefore some connection between metabolism and maturity (as shown by peroxidase tests) was suspected Peschel (1930) believes that the metabolism of leukæmic cells is similar to that of young tissue cells. Hoesch (1940) found an increase in the phosphorus metabolism in leukæmia and suggests a diet rich in phosphorus as a form of therapy. Fleischmann (1933) found that blood cells, unlike cancer cells, do not produce *lactic acid. It is probable that the increased basal* metabolic rate in patients with leukæmia (Grafe, 1927; Weil and Aschkenasy, 1938) has some connection with increased metabolism of the bone marrow

Bock and Felix (1939, 1940) evolved a new method in order to avoid the errors resulting from the admixture of blood with the marrow material, which is almost inevitable in the estimation of *the metabolism per ml of sternal marrow.* They relate oxygen consumption to the content of purine nitrogen and express oxygen *consumption in mg or g. of purine nitrogen.* In the normal marrow they found 23 cmm. of oxygen was used per hour by each ml of marrow, i e, 12 cmm. oxygen per mg purine nitrogen The total values for nitrogen were 18–35 mg. in the healthy subjects and the purine nitrogen values were 1·3–4 mg per ml The ratio of total nitrogen to purine nitrogen varied between 1 : 7—1 : 19. Contrary

to Schretzenmayr and Bröcheler (1936), Bock and Felix (1939) found that not only the red but also the granular series took part in oxygen consumption. They investigated various diseases and their results are as follows: low purine nitrogen values were found in panmyelophthisic anæmia and allied states (0 5 mg per ml.) in hypoplastic marrow processes, multiple myelomatosis and in some of the leukæmias. Certain other leukæmias showed an increased oxygen consumption. In septic processes with moderately increased numbers of normoblasts and a slight shift to the left, oxygen consumption was about twice normal.

In pernicious anæmia oxygen consumption depended on the extent of the megaloblastosis in the marrow. The oxygen consumption was 62 cmm. per hour per ml of marrow when there were many promegaloblasts, 61 cmm. when there were many megaloblasts, 38 cmm when there were many megaloblasts with partial maturation arrest and 25 cmm. when half the cells were megaloblasts With parenteral liver therapy, oxygen consumption decreased, and the lowest level coincided with the reticulocyte crisis in the marrow. Oxygen consumption was also very high in hæmolytic jaundice and in Lederer's anæmia. This demonstrated the importance of the primitive erythroblasts and megaloblasts for the oxygen consumption of the marrow in pathological cases.

Bréza (1926) examined the bone marrow of guinea-pigs and found oxygen consumption was 0 0792 ml per g corresponding to 1 53% of the entire respiration of the animal Like Michelazzi (1938) he found a considerable increase in the internal respiration of the marrow, sometimes two or threefold, when anæmia was induced by repeated bleedings Orr and Strickland (1938), working on rats, found values which corresponded to those for malignant cells and for the renal cortex

Bock and Felix (1939) could not establish any definite relationship to the protein content of human bone marrow Keilhack (1938) suggested that the protein content is characteristic for each individual disease Bock and Felix found high values for total proteins in the marrow in those diseases where protein production was disturbed, such as the nephroses and multiple myelomatosis.

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CHAPTER XIX

THE DEFENCE REACTION OF MARROW CELLS

DIRECT and indirect tests of the function of marrow cells have been studied (Leitner, 1938; Leitner and Gugelot, 1938) Observation of the blood and marrow reactions after injections of foreign material, e.g., tuberculin (Leitner and Gugelot) forms the indirect method of approach Hoff and Linhardt (1928), Zundel (1936), Stahel (1936) and Moeschlin (1945) used pyrifer or other bacterial matter Hadorn (1944) employed the method of the artificial abscess produced by turpentine. Usually the result is a shift to the left in the marrow. Like Markoff (1936) we noticed an increase of myelocytes in the marrow in a patient with caseous tuberculosis of the lymph glands after an injection of tuberculin The blood and marrow reactions which occur following the administration of hormones may be regarded as a form of indirect examination of the function. Frey and Hagemann (1921) reported lymphocytosis following a dose of adrenalin which was not confirmed by Walterhöfer (1921), Billigheimer (1921) and others. Thaddea (1938), Behr (1939) and others, however, consider that this type of leucocytosis does not depend on a redistribution of the cells, but is a true increase in the release of cells from the leucopoietic marrow portion

The testing for phagocytosis in the marrow is the most important direct examination of function The references in the literature on experiments with phagocytosis carried out on the blood have been collected in our paper on opsonins in animal experiments (Leitner, 1938). Bartel and Neumann (1906), Bergel (1921), and Doskočil (1923) recorded a high degree of phagocytic activity in the lymphocytes, but like other authors we have observed it only in granulocytes. Ponder and Flinn (1926) found that there is no relationship between the form of the nuclear structure and the phagocytic activity of the granulocyte On the other hand like Goto (1931) and Ehrich (1935) we found that mature cells are more actively phagocytic than immature ones. The eosinophils also are phagocytic, though to a lesser degree than the neutrophils (Jacobsthal, 1923; Goto, 1931, Leitner, 1938, Tobler and Buser-Pluss, 1942) Mast cells do not exhibit phagocytosis (Goto). We found that monocytes show a fair degree of phagocytosis Tobler and Buser-Pluss (1942) confirmed this recently in an interesting case of neutropenia In our animal experiments we found that the leucocytes of guinea-pigs, obtained from peritoneal fluid, artificially produced by an injection of Aleuronat were eminently suitable for experiments with phagocytosis In the human, Leitner and Gugelot (1938) were the first to test phagocytosis on the marrow in cases of

Pelger's anomaly. We found marked phagocytic activity in the mature granulocytes and less in the immature forms (Fig. 192). Markoff (1937) injected a suspension of carbon particles intravenously into a patient and found them deposited in the phagocytic reticulum cells of the marrow. Using the same method du Bois (1938) proved the absence of phagocytosis in plasma cells in a case of multiple myelomatosis. Jombres (1940) "blocked" the reticulo-endothelial system with Indian ink injections and was able to produce transient agranulocytosis.

Galinowski (1939) used the method of examining phagocytic activity in infected marrow *in vitro*. Granulocytes disappeared more rapidly than lymphocytes and erythroblasts. The speed with which granulocytes were destroyed depended on their affinity to the pathogenic organisms. The way in which phagocytosis proceeded depended on the variety of the organism and on the number of nucleated cells. Kestermann and Vogt (1939) carried out *in vitro* experiments on the sternal marrow of diabetic patients, and on the blood in patients with tuberculosis, pneumonia, malignant disease and nephritis. They found less vigorous phagocytosis in these conditions. Domenighini and Braghin (1938) recorded phagocytosis by megakaryocytes and states that they ingested leucocytes, normoblasts, lymphocytes and, more rarely, monocytes. In our experience we have not seen phagocytic megakaryocytes, and we believe that these authors misinterpreted a chance superimposition of cells ("pseudo-phagocytosis"). Tobler and Buser-Pluss (1942) found normal marrow phagocytosis in a child with neutropenia. Nordenson (1938) investigated 3 cases of reticulosis. By sternal puncture he injected solutions of Indian ink on several successive days and found 10% of the marrow reticulum cells (*i.e.*, 0·5% of all marrow cells) took up the particles of Indian ink. This corresponds to the number of phagocytic reticulum cells normally found in the marrow.

We have investigated phagocytosis by sternal marrow cells in 79 patients. In 60 cases we used the same strain of human tubercle bacillus, grown on Löwenstein's egg medium, in the same concentration. In 14 cases we used a strain of staphylococcus aureus grown on agar-agar. The sternal marrow material was mixed with sterile 3·8% sodium citrate solution, and then the bacterial emulsion was added to a concentration of 1 in 10. The test tube was incubated for 1 hour and the serum pipetted off. The cellular portion was placed on a slide, dried, fixed with absolute alcohol and stained with Giemsa's or Ziehl-Neelsen's method. The results are shown in Table 22 (p. 414).

[illegible] more vigorous phagocytosis than [illegible] *release of mature leucocytes* [illegible] be a purposeful process, the *body attempting to allow* [illegible] competent to take their share in

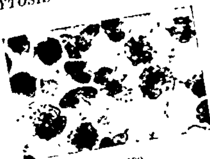


Fig. 190



Fig. 189.



Fig. 191.



Fig. 192



Fig 193



Fig 194

Fig 189 Phagocytosis of staphylococci in sternal marrow (× 500)

Fig 190 Phagocytosis of staphylococci in sternal marrow in neutropenia (× 1,000)

Fig 191 Phagocytosis of staphylococci in bone marrow (× 500)

Fig 192 Phagocytosis of tubercle bacilli in bone marrow from a case of Pelger Huët anomaly (× 1,000)

Fig 193 Phagocytosis of tubercle bacilli in bone marrow (× 1,400)

Fig 194 Peroxidase reaction in normal bone marrow (× 500)

TABLE 22

Phagocytosis of Marrow Cells

Disease	Number of cases	Phagocytosis
Chronic myeloid leukæmia	2	Normal
Chronic lymphatic leukæmia	2	Normal
Acute myeloid leukæmia	2	Reduced
Panmyelopathy and panmyelophthisis	5	Reduced in 4, almost normal in 1 chronic case
Acute agranulocytosis	1	Reduced
Thrombocytopenia	5	Reduced in 2, normal in 3 cases
Cirrhosis of the liver	2	Normal
Parenchymatous jaundice	3	Normal
Carcinoma	10	Normal
Pulmonary tuberculosis	18	Normal in 11, slightly reduced in 4, slightly increased in 3 cases
Tuberculosis of lymph glands	1	Normal
Measles	1	Normal
Septic sore throat	1	Normal
Nephritis	4	Normal
Hodgkin's disease	2	Normal
Rheumatoid arthritis	3	Normal
Ovalocytosis	2	Normal
Boeck's sarcoidosis	8	Normal in 4 cases, slightly increased in 4 cases
Addison's disease	1	Normal
Secondary anæmia	6	Normal
Total	79	

the defence mechanism, to reach the blood stream. A proportion of reticulum cells also showed good powers of phagocytosis. Reduced phagocytosis of cells with disturbed maturation was shown in the acute types of myelopathy such as acute agranulocytosis, panmyelopathy, panmyelophthisis, and acute leukæmia. The claim of Kestermann and Vogt (1939) that phagocytosis was reduced in tuberculosis could not be confirmed. In the case of other diseases the variations were too slight to allow us to form a definite opinion We have not observed phagocytosis in erythroblasts and megakaryocytes Stockinger (1933) and Leitner and Gugelot (1938) judge the maturity of cells by the peroxidase reaction As far as individual diseases are concerned, we have not yet come to any final conclusions because only marked deviations from normal maturity as shown by the peroxidase reaction are of value, and these only occur in acute myelopathies (Fig. 194)

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CHAPTER XX

TISSUE CULTURE OF MARROW CELLS

EXTENSIVE investigations have been carried out on cultures of cells from the bone marrow. Lambert (1912), Foot (1913), Barta (1926), Erdmann, Eisner and Lasar (1926), de Haan (1927), Lang (1928), Lewis (1928), *Maximow (1928), Sabin (1928)* and *Chlopin* (1934), examined bone marrow from various animals. Hirschfeld (1927), Meier, Posern and Weitzmann (1937), Bichel (1939), Osgood and his colleagues (see Osgood, 1938, 1939), Rich, Wintrobe and Lewis (1939), Fieschi and Astaldi (1941) worked on human bone marrow. *Growth* as *well* as movement of cells has been studied. Rich, Wintrobe and Lewis distinguished myeloblasts and lymphoblasts on the basis of the *difference of movement of the cells. Meier,* Posern and Weitzmann made tissue cultures from marrow from the ribs. On the first day they observed the emigration of granulocytes, later on they studied the development of fibroblastic tissue, in the meshes of which various round cells were found. There were also *large cells, almost 20–25 times the size of other marrow cells, with* coarse granulation apparently due to lipoid storage. Weitzmann and Posern (1937) succeeded in growing marrow reticulum cells from sternal puncture material. They noted a tendency to lipoid storage and to phagocytosis, and they distinguish the following stages of the development of marrow culture .—

Stage of emigration On the 2nd to 4th day emigration of granulocytes occurs

Stage of phagocytosis. From the 5th day onwards, cells with dark cytoplasm and a tendency to phagocytosis appear, which correspond to the enormous cells already mentioned.

Stage of network formation Smaller cells with round nuclei *appear and at the periphery a wide mesh-work can be recognized*

Stage of transformation with fibrocytic elements It is uncertain how far this fibrocytic tissue maintains the characteristics of marrow.

Osgood and his colleagues have modified the technique of marrow tissue culture and succeeded in prolonging the life of preparations to more than 50 days *They found that the average life of the leucocytes* was 60 hours. In cultures they develop from the immature cells and remain phagocytic. This also applied to the segmented polymorphs, which are found 20–40 days after the culture was first prepared. Eosinophils live for 8–12 days and basophils for 12–15 days. *The lymphocytes live for as long as the culture can be* kept alive. Promyelocytes show intensive karyokinesis, but lymphocytes divide by amitotic processes Fieschi and Astaldi (1946) published a monograph with numerous good photo

micrographs, made from fresh preparations, smears stained by the May-Grunwald-Giemsa method, and histological sections The cultures of human sternal puncture material from 30 healthy subjects were kept alive for 10–12 days. In cultures, 7–8 days old, the myeloid cells showed karyokinesis at the stages of myeloblast (of Ferrata's nomenclature) and promyelocytes and to a lesser degree at the stage of myelocytes. The majority of these cells do not reach full maturity, but degenerate. A small proportion develop into segmented polymorphs. Eosinophils are more hardy and multiply more readily. Their number increases progressively in the culture (20–30–40%) There were also large histiocyte-like eosinophil cells, which could not be classified with certainty. These results agree fairly well with our clinical and hæmatological observations of the "dissociated marrow reactions."

Fieschi and Astaldi (1946) consider that the hæmocytoblasts probably develop from hæmohistioblasts. It is, in any case, most unlikely that the hæmocytoblasts which were found on the 5th to 6th day had been present from the beginning of the culture preparation They showed no degenerative changes Erythroblasts could be maintained in the culture, but they survived usually for 2 or 3 days less than the granuloblasts. After 3 or 4 days there were young proerythroblasts in mitosis, and they developed into basophilic normoblasts. These, and even more so the polychromatic forms, developed into orthochromatic normoblasts. Proerythroblasts cannot mature into any other series but the erythroid one. Cells from megaloblastic marrow in cultures maintain their characteristic loose chromatin structure for a long time, later they become entangled among the prolific histiocytic elements Cells from the marrow of patients undergoing liver therapy show the same features, though less marked, and in these cultures the histiocytic reaction is not nearly as prominent Lymphocytes remained in the culture without any alteration Megakaryocytes were very hardy and were easily maintained in culture, but they showed neither platelet formation nor phagocytosis The histiocytic elements were easy to culture and kept their phagocytic activity.

Weitzmann (1941) carried out marrow tissue cultures in various diseases He established two types in the case of rheumatism, a round cell and a fibroblastic type The latter occurred in the proliferative types of arthritis His cultures of cells from Hodgkin's disease favour the theory of the neoplastic nature of this disease, though the evidence was not conclusive

Timofejewsky and Benewolenskaja (1927, 1929) cultured tuberculous tissue and studied the development of the specific tissue changes

There are still vast problems in this field, which must await further study and observation Results obtained so far are of great

interest and have contributed much towards the understanding of the origin of cells.

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CHAPTER XXI

SUMMARY

The results of marrow biopsies are recorded, based on an experience of more than 2,700 sternal punctures in more than 920 patients. Marrow biopsy is of immense practical value in the diagnosis of disorders of the blood and of many other diseases. It has also produced much valuable knowledge providing new points of view for many hæmatological problems. The question of the origin of the blood cells has been largely solved by its use. When assessing marrow findings, not only the morphological picture of its composition is of importance, but also the number and form of mitoses. They tell us about the intensity of proliferation.

In the untreated case of pernicious anæmia, the megaloblastic marrow is characteristic. Neutrophils, megakaryocytes and reticulum cells also show typical changes, such as giant forms and hypersegmentation. Pernicious anæmia, therefore, is a real panmyelopathy, and a deficiency disease. Serial punctures enabled us to observe the rapid transformation of promegaloblastic and megaloblastic marrow into normoblastic marrow and a return to normal of the granulocytes under the influence of liver therapy. There are minor distinctive hæmatological features about the individual megalocytic anæmias of the pernicious type (sprue, diphyllobothrium anæmia, pernicious anæmia of pregnancy). The finding of megaloblasts in the marrow indicates that they are related in some way to one another.

In idiopathic hypochromic anæmia and in some secondary anæmias, hæmorrhagic anæmias and others, hyperplasia of erythropoiesis is seen, mainly made up by normoblasts. In certain other symptomatic anæmias, such as the anæmia of nephritis, toxic anæmias from benzol, gold, sulphonamides, etc., anæmias of severe infections, such as tuberculosis and the anæmia of malignant disease and in leukæmia, there is hypoplasia of erythropoiesis. Hyperplastic reactions suggest a more favourable prognosis. The response to treatment is often shown by an increase of normoblasts. Hæmolytic anæmias, comprising hæmolytic jaundice, Lederer's anæmia, Cooley's erythroblastic anæmia and toxic hæmolytic anæmias, are accompanied by a particularly marked increase of erythroblasts. The primitive forms, proerythroblasts, early basophilic normoblasts and late normoblasts take part in this increase. Marrow hyperplasia frequently causes skeletal changes.

Erythræmic myelosis (di Guglielmo's disease) is a disorder of the red series analogous to leukæmia. It is characterized by enormous erythroblastic hyperplasia with a pathological type of regeneration

(anaplasia). In polycythæmia vera, all three marrow system (red, white and megakaryocytic) are hyperplastic. In erythrocytosis the *normoblasts only are increased.*

In the investigation of leukæmia, sternal puncture is particularly valuable in the diagnosis of the aleukæmic forms. It allows important *conclusions to be drawn in* connection with the problem of cell origin and the question of extramedullary hæmopoiesis. Abnormalities of cells and of cell division, which are observed in chronic, and even more pronounced in acute myeloid leukæmia, indicate a severe irreversible disturbance of blood formation. In the case of lymphatic leukæmia, sternal puncture is also helpful In the initial stages, an increase of lymphocytes occurs, which, in advanced cases completely dominates the marrow picture. A case with localization confined to the marrow is also described. It seems certain that acute lymphatic leukæmia may occur in adults also. In acute leukæmia with undifferentiated stem cells the distinction between myeloid and lymphatic leukæmia is *often impossible, even with the* help of marrow biopsy.

Especially interesting are the results in cases of agranulocytosis *(neutropenia), panmyelopathy and panmyelophthisis. Sternal* marrow biopsy enables us to differentiate between moderate cases, depending on a low grade maturation arrest and disturbance of the release mechanism, and severe cases *with advanced maturation* arrest (promyelocytic marrow), and cases with aplastic reaction (poorly cellular marrow with reticulum cells). Pure cases of agranulocytosis are relatively rare; other systems are usually involved The systems do not usually react in a parallel way, but are mostly dissociated, *e g*, if one series shows aplasia, another may show maturation arrest, and yet another may even show hyperplasia. In isolated cases of agranulocytosis, the neutrophils are frequently the only cells to suffer damage, while the eosinophils are normal or hyperplastic. Although extreme caution is indicated when prognosis is to be based on marrow findings, sternal puncture has proved its value in the investigation of myelopathies

Interesting points have been made in the assessment of various forms of leucocytosis and in the marrow reactions in infectious *diseases* Frequently infectious or toxic shifts to the left are seen in the granular series, less often generalized inhibitions of the marrow occur, such as in undulant fever and in some forms of tuberculosis *The so-called toxic granulation* of leucocytes is not a peripheral phenomenon, but originates in the marrow as a disturbance of maturation. In Pelger-Huët's familial nuclear anomaly of leucocytes *there is a shift to the left in the* [illegible] The typical nuclear structure with coarse lumping of [illegible] also appears in the primitive cells ([illegible] myelocyt[illegible] indicates a consider*able medullary* [illegible] of leuco[illegible]

The interpret[illegible] forms [illegible] cytopenia has been

advanced and diagnosis and therapy have thus benefited by marrow biopsy. Splenectomy is indicated in the thrombomyelopathic forms with maturation arrest only, but not in thrombomyelophthisic forms with aplasia of the megakaryocytic systems. In essential thrombocytopenic purpura and certain symptomatic (drug) thrombocytopenias the number of megakaryocytes is usually normal or even raised, but there are morphological (shift to the left, signs of degenerative changes in the megakaryocytes) or functional (decrease of platelet-forming megakaryocytes) disturbances of maturation. In infectious or toxic thrombocytopenia, hypoplastic or aplastic marrow reactions are seen quite often. The megakaryocytes suffer disturbances of maturation in thrombasthenia and in thrombopathy. They are frequently increased in thrombocythæmia.

Sternal puncture has advanced the knowledge of the reticuloses, the storage diseases and Hodgkin's disease, by providing facilities for the observation of reticulum cells and storage cells in the marrow. In diseases of the liver and in disorders of the endocrine glands, diagnosis cannot be made by sternal marrow biopsy. In malignant disease with bone marrow metastases, tumour cells are found by sternal puncture in about 10% of the cases.

In certain infectious diseases the pathogenic organism may be demonstrated by direct smears of the bone marrow; also the organisms may be cultured from the sternal marrow even in the absence of a positive blood culture.

Investigations into the metabolism of marrow and blood cells, and into their defence reactions and also tissue culture of marrow cells have produced results important in both clinical and academic medicine. Though many problems still await investigation and solution, marrow biopsy has proved its value in diagnosis, prognosis and even in therapeutics and has already given invaluable aid in the elucidation of many hæmatological problems.

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